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CLINICAL AND EXPERIMENTAL

VALUE OF THE LEUCOCYTE FRAGILITY TEST IN THE PROGNOSIS OF PNEUMONIA*

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CONSIDERABLE attention is given by the clinician to the total leucocytic count and to the estimation of the percentage of the various cells in the blood as an aid in the diagnosis, treatment, and prognosis in certain conditions. While exceptions to expected figures are not infrequent, a high leucocyte count when accompanied by a proportionate increase in the polymorphonuclears is interpreted as an indication of high resistance. Conversely such conditions which *should* be accompanied by a high leucocytic count, when they fail to be so accompanied tend to end fatally.

If the number of leucocytes alone is taken as the index of resistance to infection, the inferences drawn must necessarily often be faulty; and any test that would tell us something *more* about the efficiency of those cells which aid in combating infection would naturally be a valuable addition. Indeed there have been many such efforts; namely, Walker's index of resistance, Wright's opsonic index, the Arneth formula, and its modifications, all aiming to secure more data from hematologic studies.

Another perhaps more valuable method of study is the determination of the so-called fragility of the leucocytes.

In 1916, Mauriac[1] described a technic for the determination of the fragility of the leucocytes, later with Carbonet and Moreau[2] two other papers were published. They proposed an index of fragility and its behavior under various conditions and diseases studied.

Mauriac was particularly interested in a phenomenon which he called

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"Oscillation of Defense." He observed a fluctuation in the curve of fragile cells coincident with certain critical stages, such as the crisis of pneumonia, the clearing of the spinal fluid in the purulent meningitis, etc. This oscillation is characterized by a diminution in the number of the fragile cells prior

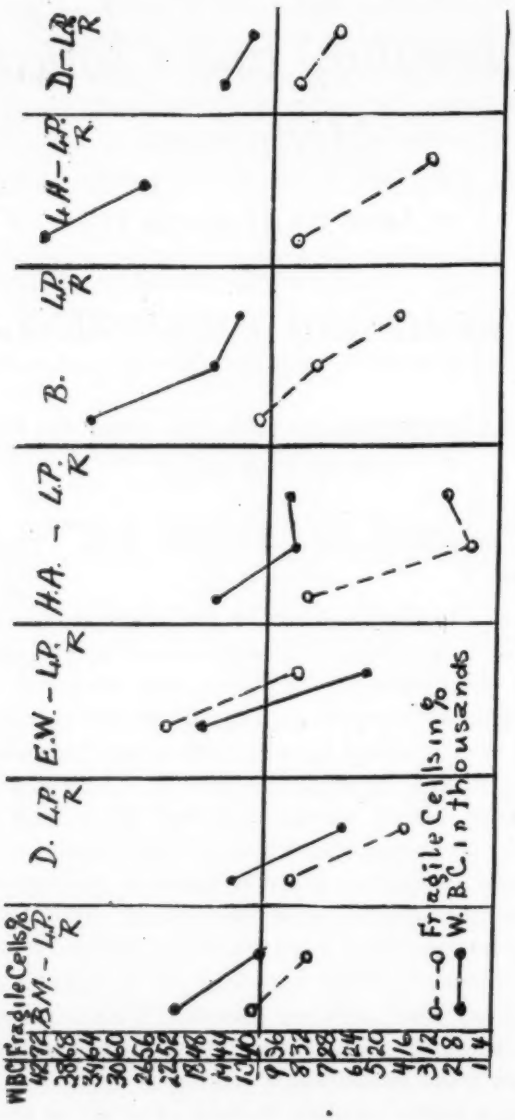

Fig. 1.

to crisis, followed by a sharp increase at the time of crisis and a posteritical fall to about their former level.

Mauriac associated this increase in the fragile cells with a rapid liberation of ferments and antibodies. While we have occasionally observed this so-called *oscillation of defense,* we are not prepared to confirm or disprove Mauriac's theory. However, we think that this increase of fragile cells may be the result of lessened demands for the delivery of new cells into the blood stream, as the disease attains its evolution and the patient recovers; in which

EXPLANATION OF ILLUSTRATIONS

Fig. 1.—Group 1. Pneumonia patients who recovered. The fragile cells are at or below the danger zone; as the disease progresses to a favorable termination the number of fragile cells decreases. The leucocytic curve parallels the fall in the fragile cells.

Fig. 2.—Group 2. Pneumonia patients who recovered. The fragile cells are below the danger zone and rise to or about normal. This series shows that the curve of fragile cells is not dependent on the leucocytic curve.

Fig. 3.—Group 3. Pneumonia patients who recovered. The fragile cells are below the danger zone. There is a rise in the number of fragile cells at crisis, and a postcritical fall. This is Mauriac's Oscillation of Defense.

Fig. 4.—Group 4. Pneumonia with complications. This group shows a rise in the fragile cells prior to or with the onset of complications.

Fig. 5.—Group 5. The effect of mercurochrome on the fragile cell count in pneumonia. A rise after the injection of mercurochrome was followed by a sharp drop. If the treatment was beneficial the fragile cells continued to fall; if it failed, the fragile cells did not fall below the danger zone and instead of continuing to drop (as in those that recovered) they rose very rapidly to their former high level.

Fig. 6.—Group 6. Cases of lobar pneumonia and miscellaneous septic infections. At the time of complications the number of fragile cells increased. The result of either surgery or specific measures, if favorable, was marked by a drop in the fragile cells.

Fig. 7.—Group 7. Patients with lobar pneumonia who died. All showed a high fragility at time of death; sometimes the fragile cells were low in the early stages but a gradual increase was observed as the patient became worse.

Fig. 8.—Group 8. Cases of influenzal pneumonia. All patients recovered. The fragile cells were low.

Fig. 9.—Group 9. Pneumonia patients died with a low fragility. Unfortunately some have but one count. No. 1 died of acute cardiac dilatation.

Fig. 10.—Group 10. Two patients with lobar pneumonía who recovered with a high fragile cell count. We have no explanation for the apparent contradictions.

case the rapid rise in fragile cells would be the *effect* of the crisis and not its cause.

Recently Sampson,[3] of the University of California, has published a comprehensive study of the subject and modified the technic so that it is scarcely more complex than an ordinary leucocyte count.

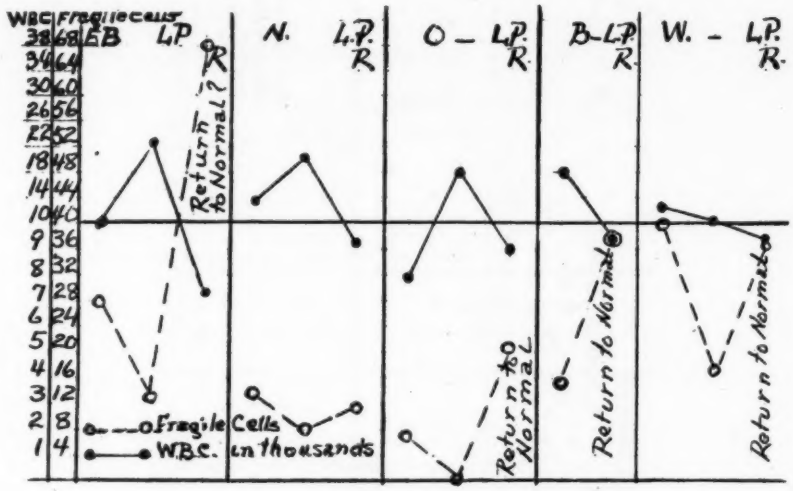

Fig. 2.

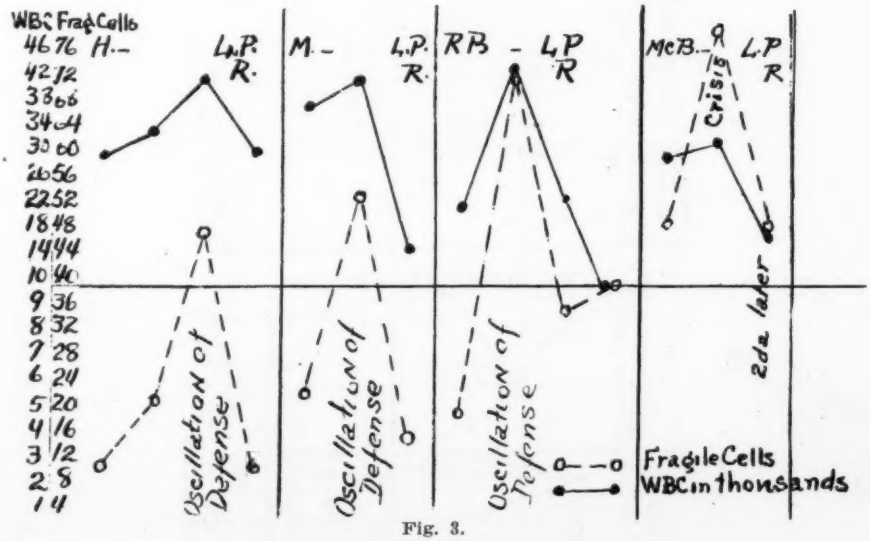

Fig. 3.

The principle of the method is as follows: the blood cells are subjected to a standard-injuring medium in the form of a hypotonic sodium chloride and sodium citrate solution. Then the blood suspension is brought to isotonicity by the addition of hypertonic saline solution and a nonvital dye is added.

With Sampson's method the differentiation between a fragile cell and a resistant cell is very sharp. The nuclei of the fragile cells appear diffusely colored by the dye and often these fragile cells are distorted. The resistant

cells remain colorless and refractile; the erythrocytes are largely removed by laking. Sampson considers that the percentage of fragile cells in the normal individual, is about 45 to 55 per cent (with 43 to 63 per cent as the extremes). He also studied the fragility of the leucocytes in several diseases.

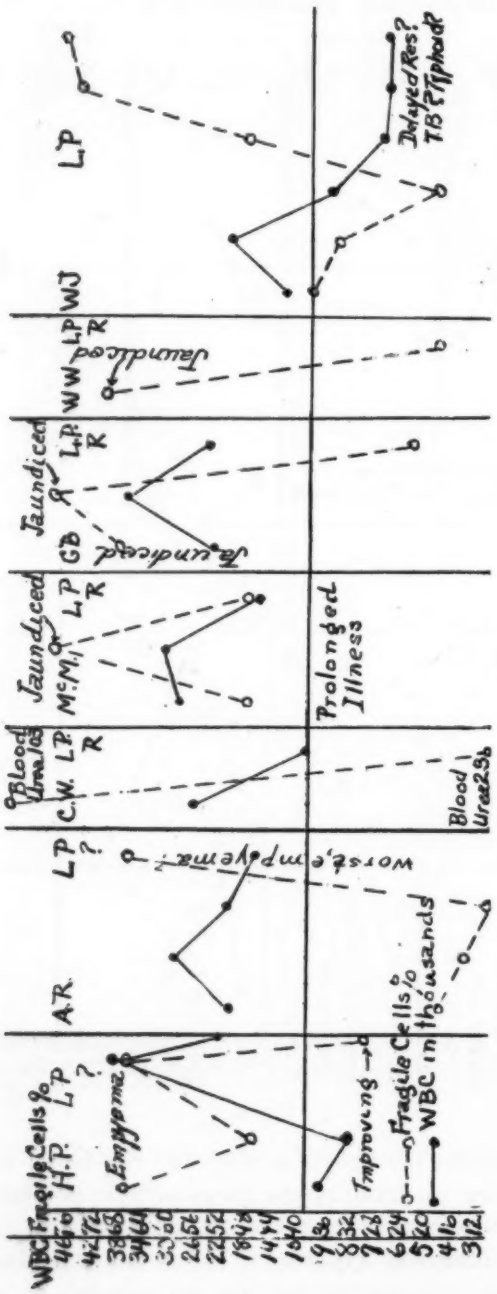

Fig. 4.

Of six patients with lobar pneumonia that he studied, four that recovered, showed a marked reduction of fragile cells and two that died, showed only a moderate reduction.

Pneumonia seemed a particularly suitable disease for this study; we were especially interested in noting the behavior of the fragile cells during its course. An effort was made to obtain as many counts as possible, especially at or near the crisis, and during convalescence. We have tried to correlate the influence of complications, treatment, and other factors which might

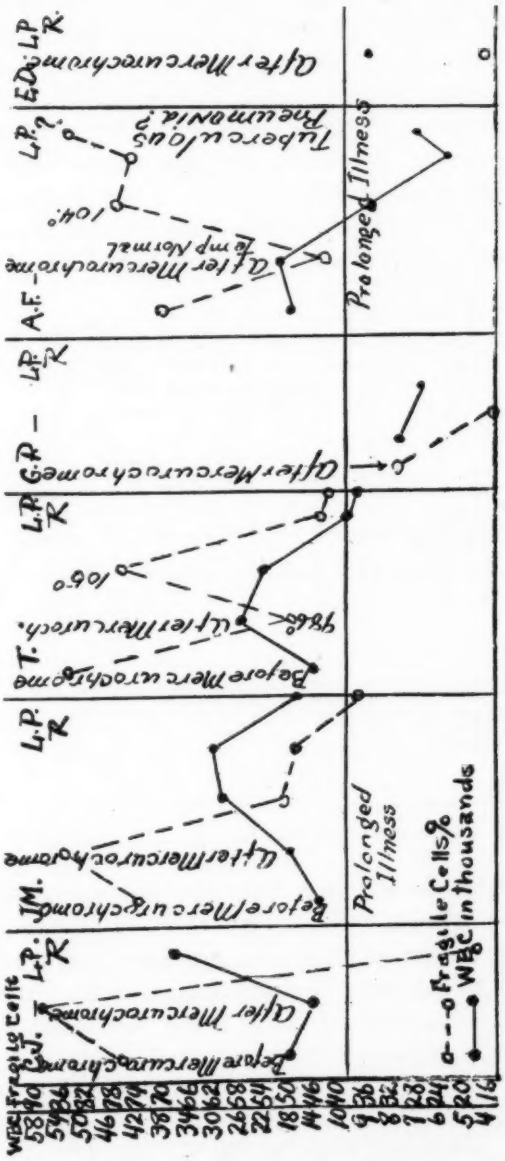

Fig. 5.

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alter the course of the infection, and as a check on our technic, a few other acute infections were studied.

Method.—A drop of blood is drawn into a leucocyte counting pipette to the mark "1," and the diluting fluid, consisting of 0.1 gm. of sodium citrate and 0.05 gm. sodium chloride to each hundred cubic centimeters of distilled water, is drawn up to the "11" mark. The pipette is gently rotated while

the fluid is drawn up. It is not shaken, but slowly rotated while the mixture is blown into a test tube, of from 5 to 8 mm. internal diameter and from 3 to 6 cm. in length, which preferably has been lined with paraffin. The test tube containing the diluted blood is corked, preventing evaporation which would cause an alteration of the concentration, and is then gently agitated for one minute to insure an even distribution of the cells throughout the solution. It is then allowed to rest undisturbed for exactly two minutes more, during which period the same pipette is washed out with water and the barrel refilled to the point "1" with 9 per cent sodium chloride solution. At the expiration of the total three minutes the 9 per cent sodium chloride is blown into the solution, the entire mixture drawn once into the pipette and then expelled into the test tube. One drop of 1.0 per cent aqueous solution Niagara blue or 1.0 per cent trypan blue is then added to the contents of the tube, which is gently shaken once or twice and then recorked. A

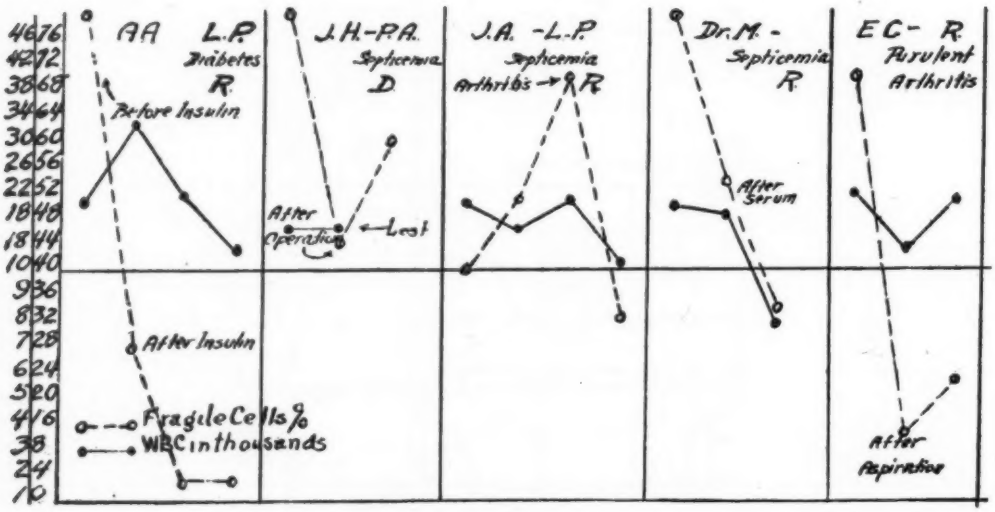

Fig. 6.

large drop of the solution is taken from the test tube, placed on a smooth glass slide and covered with a No. 0 or 1 thickness cover slip which should not contain marked ridges or other irregularities and should measure from 1.5 by 1.5 cm. to 2.5 by 2.5 cm. The preparation is then rimmed with melted paraffin. The relative number of stained and unstained granular and non-granular white cells is counted in the proximal, central, and distal portions of the preparation, running from one margin to the other of the cover slip.

Certain precautions need to be emphasized. Excessive trauma in collecting and manipulating the specimen should be avoided; the entire procedure should not consume more than fifteen minutes and therefore should be done at the bedside. While a dye of low toxicity should be used, such as trypan blue or Niagara blue, *vital* stains should be avoided, as these also penetrate the still living or resistant cell. The diluting solutions have to be changed from time to time and should be kept in the ice chest and free from gross bacterial contamination.

The following experiments convinced us that under the conditions of study, the stain or fragile cells are either injured or dead cells.

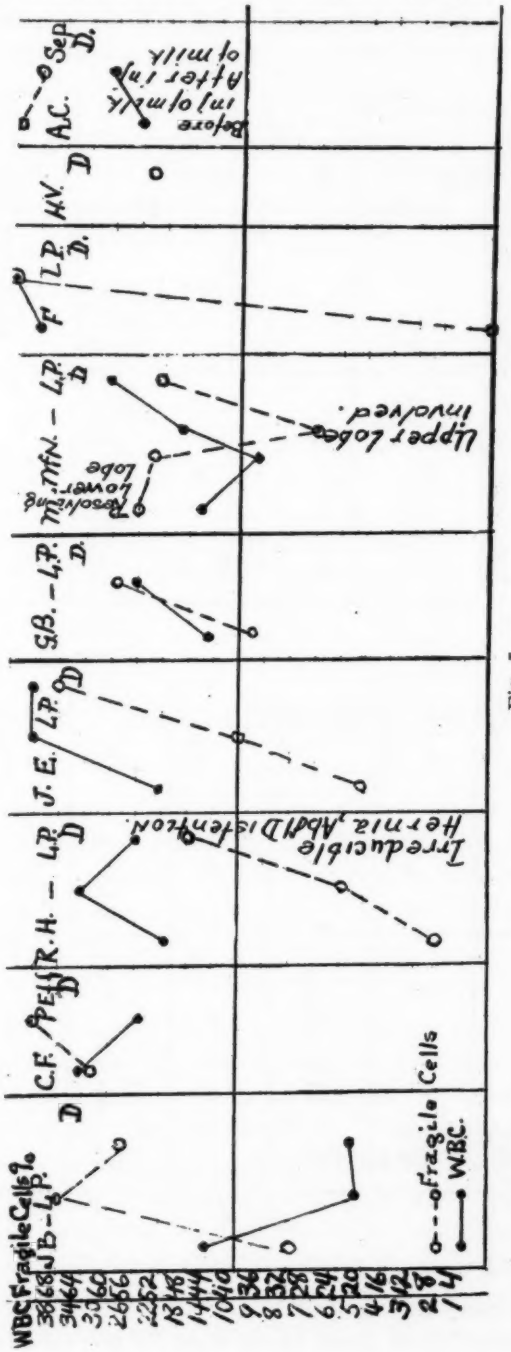

Fig. 7.

Experiment No. 1.—

Spinal fluid from a case of purulent meningitis showed:

a. Before boiling—10 per cent fragile cells.

b. After boiling—91 per cent fragile cells.

It is safe to assume that the effect of heating the spinal fluid to the boiling point is to kill all the cells. The rise of fragile cells after boiling justifies the assumption that the so-called fragile cells are injured or dead cells.

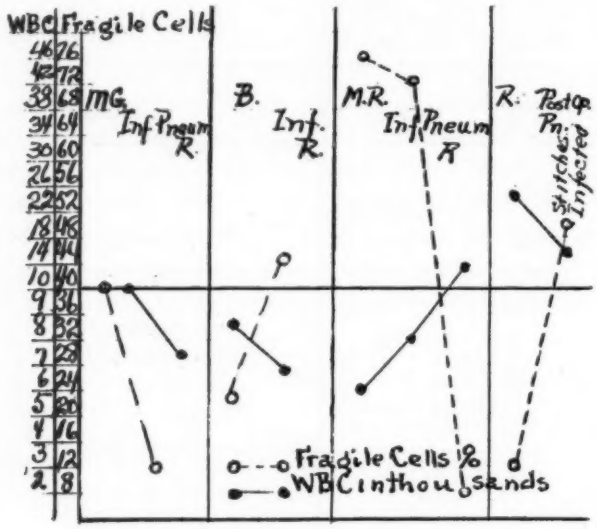

Fig. 8.

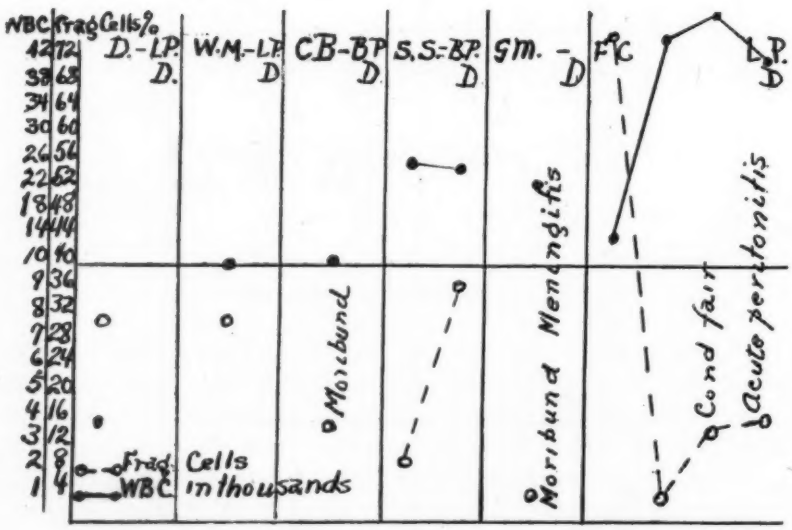

Fig. 9.

Experiment No. 2.—

Pus from a knee-joint showed 12 per cent fragile cells five hours after removal; twenty-four hours later, there were 36 per cent fragile cells; forty-eight hours showed 64 per cent and there were 88 per cent in seventy-two hours. At this time there was so much disintegration that it was difficult to separate the cells. The increase in the percentage of fragile cells was therefore parallel with the increase in cell death.

Experiment No. 3.—

The opsonic index done at the forty-eight-hour period showed that 68 per cent of the cells were no longer phagocytic. This figure corresponded very closely with the 64 per cent fragile cells for that period.

Experiment No. 4.—

The motility of the leucocytes was studied by the method of Florence Sabin. Distinct motion was observed in some cells at the end of forty-eight hours, corresponding with the opsonic index and the percentage of fragile cells for that time.

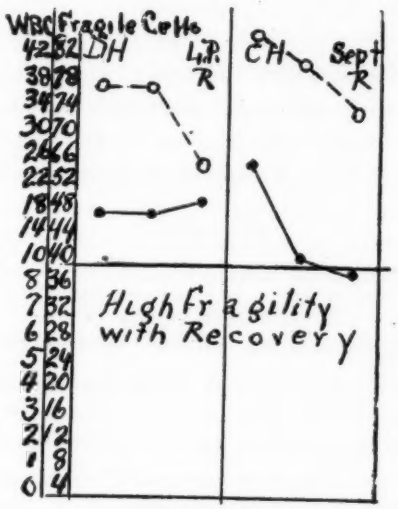

Fig. 10.

In recording our results with this test in pneumonia patients, we have arbitrarily called anything above 40 per cent fragile cells within the danger zone. The results obtained with this test, we hope to make clear by the accompanying charts.

SUMMARY

We believe that the study of the fragility of the leucocytes is of interest and value in the course of some acute infectious diseases. Like many other tests its findings are not *always* conclusive. It should never be considered by itself but along with other studies.

It has been observed that the fragile cells increase prior to complications; that a high fragility is almost always associated with either a stormy course or with a bad prognosis, particularly if accompanied by a normal or low leucocytic count. The curve of fragile cells does not depend on the leucocyte curve or necessarily correspond with subjective symptoms. In most cases this independence was gratifying, as it was a better indication of the outcome.

A single high fragile cell count is of little significance, especially in the beginning of the disease. Treatment or some other factor may change the curve of the fragility of the leucocytes, and the outcome also. We were

able to study the influence of mercurochrome in pneumonia. When it was beneficial there was a slight rise in the fragile cell count followed by a sharp fall; thereafter the percentage of fragile cells remained low. Other times, when mercurochrome was of temporary benefit only, there was a temporary tumble in the number of fragile cells; and thereafter the number of fragile cells increased rapidly. Drainage in infections, administration of sera in septicemias, of insulin in a lobar pneumonia complicated by diabetes, all produced a drop in the fragile cell count.

We must refrain from drawing any conclusions because our series is small—only fifty-five cases were studied—and our clinical observations have not been as complete as we would have liked to have had them. It seems necessary to determine the percentage of fragile cells within the limits of safety, for *each* condition in which the test is applicable. In this connection, for *pneumonia,* under our conditions, anything above 40 per cent is within the danger zone. Mauriac's "Oscillation of Defense" which was observed in a number of cases has been discussed elsewhere in this paper.

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THE ORGANIZATION OF A METABOLISM LABORATORY*

By William H. Stoner, A.M., Ph.D., Philadelphia, Pa.

THE organization of a metabolism laboratory should be planned by the clinician and the laboratorian, preferably a biochemist, who are to have charge of the department of metabolism. Very rarely this dual combination of metabolic clinician and biochemist is found in one man. By study of the organizations of, and visits to, similar departments and by frequent conferences, they should draft and redraft their plans until a satisfactory one is evolved.

It is assumed that a hospital planning a department of metabolic diseases has thoroughly equipped and manned general laboratories of chemistry, pathology, bacteriology, serology, roentgenology, animal experimentation and necropsy, and that these services are available to the metabolism department for the usual routine examinations and experiments.

In addition to these general laboratories there should be established, in an efficient department of metabolic diseases, a departmental laboratory—chiefly chemical and research—which should be so equipped and manned as to be able to supply any service requested by the clinical departmental staff, including emergency service at any time of the day or night,—week days, Sundays, and holidays. If the general laboratory of the hospital does not include departments of physics, physiology, electrocardiography, x-ray, glass-blowing, and machine shop, they must be provided for in the departmental plan.

On account of the emergency nature of a great portion of the work of a metabolic laboratory, it is quite important that the laboratory be located conveniently near the clinical department, certainly under the same roof and preferably in an adjoining room. Blood and urine sugar and plasma CO_2 reports should be forthcoming in emergency cases in fifteen to twenty minutes after taking samples.

The size of the laboratory depends upon the size of the clinical department. The usual fault in planning such a laboratory is to allow too small, rather than too large, a space. Usually within a very few years after the establishment of such a laboratory it is found to be inadequate in size, equipment, and personnel. For the usual routine work conducted by the less trained members of the staff, one large, airy, light room should be provided. For the chemists, research clinicians, food analysts, gas analysts, etc., specially equipped, more private, and smaller rooms should be available. At least two or three rooms should be fitted for general research work for additional workers not initially contemplated. Special provision should be made

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for instructional purposes since instruction in all laboratory matters, of nurses, patients, visiting students and internes should be conducted in the laboratory. Such arrangements should include a suitable assembly room equipped with blackboards and demonstration table, thoroughly equipped with gas, hot and cold water, drain, blast, vacuum and electricity. Charts, diagrams, models, etc., should be available. Office space should be provided for the conduct of records and the preparation of manuscripts.

In charge of all administrative matters and of routine and research laboratory work should be a biochemist, Ph.D., M.D., or both, thoroughly trained in general physiology, including animal experimentation, physiologic chemistry, physics, mathematics, German and French, and having considerable experience in practical hospital routine laboratory work, especially blood and urine chemistry. He should also have had a thorough training in food analysis and in food and animal calorimetry.

Under the direction of the biochemist should be one or more junior chemists of similar but less complete training and experience, who should perform the analyses suggested by the biochemist in the departmental researches.

In addition to the biochemist and his chemically trained assistants there should be provided one or more technical assistants for the performance of the more frequent determinations. In regard to the nature of the training of these technical assistants, there is much difference of opinion. Experience shows that the less training they have had in chemistry, physics, or medicine the better technical experts they become. Many regularly trained laboratory technicians consider their superficial training an adequate one for the more advanced work, even the research work of those in higher stations in the personnel. The best routine chemical work is done by high school boys with no technical training except in the very particular tests for which they are responsible. This necessitates rigid supervision by a chemist of the solutions used in the determinations and of the results obtained.

In addition to the personnel outlined, one or more clerks should be provided so that the more valuable time of the chemists is not lost in longhand writing of their results for publication or the time of the technicians lost in the cataloging and filing of results. In general, in an organization of any sort the most economical practice is to assign duties to the lowest paid member of the staff who can properly perform them. For this reason it is well to have at least one member of the personnel an unskilled laborer, and it is surprising how much of the less complicated operations of a laboratory can be performed by this employee. In the same way, after some time, the technician although formerly untrained is able to prepare the solutions used and to recognize defects arising in the work which at first required the services of one of the chemists.

There are two common errors in the initial equipment of laboratories, deficiency and excess. With the usual hospital appropriation for this purpose, however, the error is usually in the former direction. It is quite rare to see a hospital laboratory equipped with expensive dust covered apparatus which has never been used. On the other hand, it is quite common to see a laboratory unequipped, or poorly equipped, for very necessary diagnostic

work. The most expensive is usually the least extravagant. An example of this in a biochemistry laboratory is colorimeters. The cheaper, mechanically poorer colorimeters are in the end much more expensive than those of higher initial cost. The same is true of the ordinary glassware as compared with the resistance types. Again, it is no economy to use a cheap chemical when results obtained will be doubtful on that account. A laboratory for the study of metabolic diseases should be equipped to perform all the usual chemical tests of the urine, blood, feces, gastric juice and other body fluids. It should also be equipped for complete food analysis of any sort including bomb calorimetry and for all the usual respiratory exchange work used in determining respiratory quotient, basal metabolic rate, etc. All the modern functional tests should be provided for, including those of the liver, kidney, stomach, etc.

The equipment should include a library containing a small but well-selected group of textbooks and journals. The medical literature abounds in instances of research work duplicated after a period of years without the knowledge of the later author. Research work in any line should be preceded by complete bibliographic research. The departmental libraries of laboratories in more remote sections should be larger than in the cities having large medical libraries.

The biochemist should be the executive head, responsible in line of authority to the chief of the Department of Metabolic Diseases of the hospital. He should be responsible for the assignment of duties to the various members of his staff. He should control the purchase and use of supplies and reagents, making recommendations for changes in personnel, and should direct the entire research of the department, clinical as well as chemical. He should establish and maintain a routine statistical research program by a complete system of records of all analyses of the department. The difficulty usually encountered in the assignment of executive duties to a scientific departmental head is that he becomes sterile scientifically and devotes too great a proportion of his time to executive duties. With proper systematization and assignment of these executive responsibilities to other members of his staff he should be able to devote at least three-quarters of his time to investigative work.

In the conduct of an out-patient department in diabetic work, it is necessary to have immediate laboratory service so that blood and urine analyses may be conducted while the patient is in the dispensary. For this reason it is essential that at least one technician be assigned to the dispensary for the analyses of samples taken at that time. Dispensary hour should be early in the morning so that blood samples may be taken before breakfast, and so that the occupations of the out-patients will be interfered with as little as possible. With such a service the clinician can prescribe dietary and insulin treatment with almost mathematical precision.

A metabolism laboratory with an organization as outlined is able not only to render adequate and prompt diagnostic and therapeutic service but also to contribute much to the knowledge of the practically unexplored field of metabolic diseases.

EXPERIMENTAL AND CLINICAL OBSERVATIONS ON THE ACTIONS AND THERAPEUTIC USES OF ETHYLISOPROPYLBARBITURIC ACID*

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In a preliminary article[1] published by one of us in 1922, it was shown experimentally that a new chemical compound, ethylisopropylbarbituric acid, which had just been synthesized in the laboratory by Lambert Thorp, possessed hypnotic properties which apparently gave marked promise for the future usefulness of the compound in medical practice. Following up this experimental work a short account of the early clinical application of the drug was published in 1923.[2] Chemically, the compound is made by condensation of urea and ethylisopropyl-malonic-diethylester in the presence of sodium ethylate at 105° C. The free acid is but slightly soluble in cold water, but its sodium and potassium salts are freely soluble both in water and in alcohol.

Recently Thorp has prepared the calcium salt of this compound. Encouraged by the favorable results of our earlier work with the substance we have continued our investigations, using more recently this newly prepared calcium salt. This is a white, sandlike powder which is sufficiently soluble in water to permit of its experimental use by intravenous injections in animals.

In order to determine its action on the respiration and circulation, we have carried out a series of experiments on dogs, the results of which have been very satisfactory. The accompanying tracings show this as may be seen by reference to Figs. 1, 2, and 3.

In Fig. 1 we have shown the action of 3 c.c. of 1.5 per cent solution on the blood pressure (mercury manometer) and respiration of a dog weighing 11 kilos. This represents a dose of about ¾ grain (45 milligrams) or the equivalent of a medicinal dose (3.5 to 4 gr.) in man. The drug here, however, was all suddenly injected into the blood by way of the femoral vein. In this case the greatest toxic action which this quantity of the drug can produce is shown immediately on the blood pressure and respiration. It is seen that the arterial pressure is at first slightly increased (as a result of the volume of the injected solution only) and that this is followed by a small, transient fall in pressure which lasts only about forty seconds, by the end of which time the blood pressure has again returned to practically its previous normal level. This shows that doses of this size have but little action on the circulatory system, and that, therefore, one would not expect any serious symptoms to arise from the circulatory organs in the ordinary clinical use of the drug.

*From the Medical Department, University of Cincinnati, and the Psychopathic Institute of the Jewish Hospital, Cincinnati, Ohio.
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Fig. 1 further shows that the dose here used has almost no effect whatever on the respiration. The rate remains approximately constant while the variations in depth are so small as to be practically negligible.

Fig. 2 shows the results of two injections (3 c.c. and 7 c.c.) on the heart and on the blood pressure. In this experiment the chest was opened under ether anesthesia and a myocardiograph was attached directly to the heart. The record obtained from the heart is shown in the upper broad tracing in which the downstroke of the recording tambour represents the systole of the heart. The blood pressure (right carotid artery) was recorded with a mercury manometer. Here it is seen that 3 c.c. of 1.5 per cent solution of

Fig. 1.

the drug injected at the point indicated by X on the tracing had but little effect on the heart, the extent of both systole and diastole remained almost unchanged. A slight fall in blood pressure was produced but in less than two minutes the pressure had again regained its normal level. Following the first injection of 3 c.c., a large injection (7 c.c.) was given. This caused a very pronounced fall in blood pressure, but recovery from it was good and in about three minutes the pressure had again risen to normal. The extent of both systole and diastole of the heart was slightly reduced, but within a few minutes the beat had again returned to approximately its normal extent and vigor. Here we see that within a space of about four minutes no less than 150 milligrams (3 c.c. and 7 c.c. of 1.5 per cent solution) of the drug were thrown directly into the circulation and yet in less than three minutes

after the last injection the heartbeat had returned to very nearly its normal amplitude and strength while the blood pressure was fully back to normal. This experiment shows that the drug has but little deleterious action on the heart muscle itself or on the circulatory apparatus in general when the substance is given in quantities more than four times as large as the ordinary medicinal dose (3.5 to 4 gr.) by stomach; and when taken by stomach, of course, the drug would pass into the circulation very much more slowly than was the case in this experiment in which the drug was injected suddenly into the femoral vein.

In Fig. 3 the remarkable recovery which the heart and circulation in general can make after enormous quantities of this drug is well shown. Here the upper tracing (myocardiogram) was taken directly from the heart itself while the blood pressure was recorded by a mercury manometer. A total of 6 grains was injected intravenously into an animal weighing 14 kilos. The drug produced a striking effect on the strength and amplitude of the heartbeat, and the blood pressure fell sharply, yet after about five minutes the heartbeat had almost returned to normal and the blood pressure was only a few millimeters below its normal level.

In other experiments it has been shown that very large doses of the drug may be injected intravenously without causing the death of the animal,* but that when death does occur it is due to central respiratory paralysis. Consequently the treatment for poisoning with the substance should consist of respiratory stimulation or artificial respiration.

When the drug is administered in therapeutic doses the action is manifested almost entirely on the central nervous system and more especially on the cerebrum. In animals, there appears to be produced an overpowering desire to lie down quietly and sleep. After moderate doses a dog can be awakened by loud sounds, shaking, etc., but if the animal is then left alone it soon lies down and goes to sleep again.

In animals, large doses may produce slight muscular twitchings or tremors. From a practical standpoint these appear to be of no importance as they do not appear in man following the therapeutic administration of the drug.

From the therapeutic standpoint, the ideal hypnotic should be a pure chemical compound, fairly soluble in cold water, of not unpleasant taste and possessed of a rapid action. The dosage should be small with a wide latitude between the therapeutic and lethal doses. It should be free from immunizing effect, should not color the urine—an indication of destruction of the red blood corpuscles—and it should be free from a depressing action

*From the therapeutic action of the drug it seems obvious that man is more susceptible to the hypnotic effects of the compound than are dogs. In the earlier reported experiments with the sodium salt on dogs, it was found that the dose (by mouth) beyond which recovery does not occur is in the neighborhood of one grain to the pound of weight of the animal. This would indicate that an average patient of one hundred and fifty pounds weight might survive a dose of nearly ten grams or one hundred and fifty grains. It seems probable however, that the fatal dose in man would be smaller than this amount, unless the stomach should be emptied very soon after the drug had been swallowed. The lower degree of susceptibility possessed by dogs, as compared with man, probably holds only in case of the higher, psychical areas of the cerebrum; and in this case, man's higher psychical development probably mainly accounts for his increased susceptibility over that of the dog. So far as the heart and the other circulatory organs and the respiratory apparatus are concerned, it is probable that no great difference exists between man and the dog.

on the heart. Finally, it should not cause the patient to have a feeling of dopiness or drowsiness upon awakening. Moreover, its action should be selective, only the highest cerebral cells being affected by even large doses.

Such a hypnotic we feel is represented by ethylisopropylbarbituric acid.

This substance is a white crystalline compound slightly soluble in cold water. Its taste is not disagreeable and its action is very rapid. It has been used by many physicians in a great variety of cases, and so far no untoward effects upon the heart, lungs, or kidneys have been noted. The outstanding feature in all cases is the ease with which a refreshing sleep is produced and without the usual feeling of lassitude and torpor on the following day. In many instances sleep was produced where other well-known hypnotics had

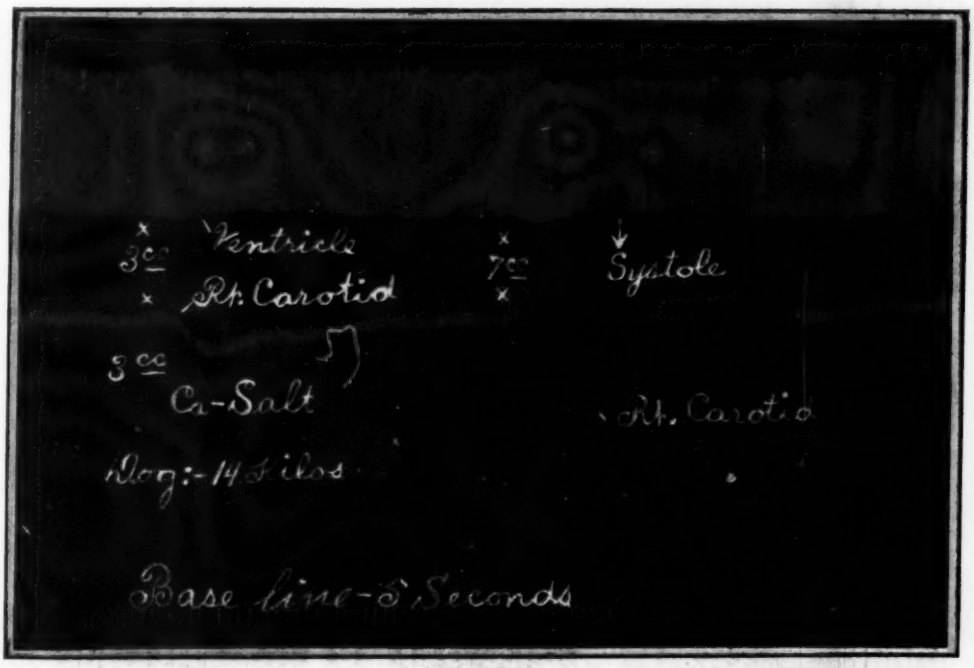

Fig. 2.

failed. The dosage has been from three and one-half to four grains, although as much as fifteen grains may be given with absolute safety. The drug has also been given hypodermically to maniacal individuals with marked sedative effect. Many surgeons have found it of particular value in combating the motor restlessness, irritability, and sleeplessness so often met with in postoperative cases.

Many individuals who are operated upon are found to have more or less impairment of the myocardium. Many of these patients require and receive hypnotics and this despite the fact that hypnotics, as a rule, are known cardiac depressants. Obviously, in such cases the choice of a hypnotic is of the greatest importance. A large number of patients all of whom showed definite myocardial involvement, and on whom various major operations had been performed, were given this drug and the effect on the heart and pulse

carefully noted. In every case without exception a refreshing sleep was obtained with no deleterious action on the heart or pulse.

Sleeplessness as a symptom is met with in a great variety of conditions, both functional and organic. Every physician knows what a troublesome symptom it is and how quickly a patient may come to rely absolutely on the use of hypnotics to produce sleep. Unfortunately, most hypnotics lose their effect quickly and the dosage must be increased. The chronic sufferers are, therefore, forced to change from one hypnotic to another irrespective of their value or danger.

Ethylisopropylbarbituric acid has been given to a great many individuals for the past three years, and so far it has been found necessary to increase

Fig. 3.

the dose from three and one-half to six grains in but very few cases. In this connection the following case report is of interest:

J. J., age fifty-eight years, was under observation at a hospital suffering from an anxiety state, the principal symptom of which was the fear of "contamination". The man was in a fearfully nervous state and suffering terriffic mental anguish. He felt that even touching his bedclothes and nightgown "contaminated" him. For about ten days previous to his admission to the hospital he had been unable to sleep. Mild sedatives and hydrotherapeutic measures were tried, but without result. He was thereupon given a nightly dose of three and one-half grains of the calcium salt of ethylisopropylbarbituric acid. During the several months that the patient was in the hospital he never failed to obtain a refreshing sleep. On several occasions five grains of veronal were given instead of the three and one-half grains of ethylisopropylbarbituric acid. The patient, however, obtained very little sleep. Ten grains of veronal were then given. The difference in the

sleep produced by ten grains of veronal and three and one-half grains of the calcium salt of ethylisopropylbarbituric acid was marked. True, the patient slept under the ten grains of veronal but it was a heavy, troubled sleep which left him dopey and drowsy the next day, and hard to control. After the exhibition of three and one-half grains of the calcium salt of ethylisopropylbarbituric acid, the sleep approached closely that of a normal sleep and the patient felt refreshed on awakening and showed no mental lassitude or torpor.

This hypnotic has also been of great value in curing individuals of the habit of taking hypnotics. This habit is prevalent among the group of patients diagnosed as neurasthenics and psychasthenics.

The effect of the older hypnotics is normally of a fleeting nature; sleep of a greater or lesser degree being obtained for one night only. The following night the neurotic individual must either take another dose or go sleepless. Furthermore, as pointed out above, in a short while the drug loses its effect unless the dosage is doubled or trebled. The harmful effects of such a procedure can be readily inferred. In addition, any individual who is a slave to a habit, no matter what its nature, suffers tremendously in one respect, namely, in loss of self-confidence in regard to that particular habit. No habit can be cured until the patient's self-confidence is restored to such an extent that he is made to feel that he is master of the habit which has enslaved him.

One of the characteristics of this new hypnotic is the fact that it not only produces sleep the night that it is exhibited but its sedative action persists so that as a rule the patient will also be able to sleep the following night without resorting to the drug. A patient who had previously found it necessary night after night to resort to hypnotics is not only greatly improved but highly elated when he finds that he is able to do without a hypnotic even for one night. This furnishes the wedge by which the patient can be convinced that he can ultimately dispense with hypnotics.

Many case reports bearing this out could be cited. However, one will suffice.

H. A., aged sixty-two, came under observation because of a severe mental depression. The history revealed that at the age of twenty-five, a partial thyroidectomy had been performed following which she became a chronic invalid. Of recent years she has been greatly troubled with insomnia and had to resort to the nightly use of veronal. Lately she has found that five grains did not produce sleep and she has had to double the dose. Three and one-half grains of the calcium salt of ethylisopropylbarbituric acid were substituted for the veronal and little by little the patient was gradually weaned away from the use of all hypnotics. The result in this case can be attributed principally to the fact that this new hypnotic not only produced a natural sleep the night the drug was taken, but also had a distinct effect the following night. Once the patient discovered that she could sleep without resorting to hypnotics, her self-confidence was reestablished and her cooperation was assured. The desired result followed naturally.

In conclusion, we wish to call attention to the fact that this new hypnotic in laboratory as well as in clinical tests, has shown superiority over the older hypnotics, both in safety and in effectiveness. The margin of safety between the therapeutic dose and lethal dose is very great. Furthermore, no untoward effect on the heart, lungs, or kidneys has been observed. In addition, it produces a sleep which approximates normal sleep very closely

and without the usual feeling of lassitude and heaviness of the following day, a condition that is so characteristic of other hypnotics.

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THE EFFECT OF HEAT ON THE HEART EXTRACT USED AS ANTIGEN IN THE WASSERMANN TEST FOR SYPHILIS*

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SINCE 1906, when Wassermann, Neisser and Bruck[1] pointed out the possibility of making use of the phenomenon of complement fixation discovered by Bordet and Gengou[2] in the diagnosis of syphilis, the problem of producing a suitable antigen has occupied the minds of many workers. It was very natural for Wassermann to use as antigen tissue extracts of a congenitally syphilitic fetus, since he considered the complement-fixation reaction in syphilis a true antigen-antibody reaction.

This theory of a specific antigen-antibody was soon disproved by Marie and Levaditi[3] who obtained positive reactions in paresis with an aqueous extract of normal liver. New light was thrown upon the subject by Landsteiner, Müller and Potzl,[4] who demonstrated that just as good results could be obtained with alcoholic extracts of guinea-pig heart as antigen as when aqueous extracts of fetal syphilitic liver were employed. Porges and Myer[5] thought that the active principle of the syphilitic antigen was lecithin. Browning, Cruickshank and McKenzie[6] found that the addition of a certain amount of cholesterol to alcoholic antigens greatly increased their antigenic value. Levaditi and Yamanuchi[7] showed that cholesterol alone has no antigenic properties.

Our knowledge was further increased by Noguchi,[8] who classified the lipoids which act as antigens, according to their solubilities in the ordinary fat solvents; alcohol, ether and acetone. He found that the active principle in the antigen is acetone-insoluble, thus proving it to be a phosphatide. He also formulated a system of titration for judging the value of an antigen.[9] He set down the following rules which must be strictly adhered to in order to obtain good results: The greater the dose of antigen used for obtaining a positive reaction with a known positive serum, the greater are the chances of picking up a doubtful reaction; at least five times the dose of antigen necessary to inhibit hemolysis with a known positive serum should be used in performing the Wassermann test. This dose must not be hemolytic by itself, and must not inhibit hemolysis with negative sera. Thus in titrating an

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antigen we must learn its three essential properties: antigenic, hemolytic and anticomplementary.

There are three kinds of antigen in general use at present: the plain alcoholic extract, the alcoholic extract with the addition of cholesterol, and the Noguchi acetone-insoluble antigen. Shuermann,[10] Sachs and Rondoni[11] used synthetic antigens which contained lecithin as a basis, and although some success was obtained with some of them, the results were not good enough to warrant their use in performing the Wassermann test. Good results, however, are obtained with the three antigens mentioned above, provided they are properly titrated.

Most laboratories make use of three different antigens as it seems unsafe to depend on the results obtained with a single antigen. The plain alcoholic extracts are the weakest antigens, and while they give no false positive results yet they may fail to pick up weakly positive sera, and it is these borderline cases which are of great importance. The cholesterinized plain extracts are the strongest antigens but may sometimes give false positives. The acetone-insoluble antigen occupies an intermediate position and is probably the safest to use. However, it is not used much because of the difficulties encountered in its preparation.

The technic for preparing plain alcoholic and cholesterinized antigens is quite simple and yet it is difficult to procure usable antigens; by using the very same method one may obtain an excellent antigen from one heart while the next dozen hearts may give worthless extracts.

In order to overcome this difficulty, and to evolve a method for obtaining a good antigen every time, Neymann and Gager[12] investigated the lipoids of heart-muscle and classified them according to their antigenic value in the Wassermann test. They extracted the various fractions of lipoids, following the method of Erlandsen[13] which divides the various lipoids according to their solubilities in ether, alcohol and acetone. In the main, their method consisted in making two extracts, a primary ether-soluble and a secondary alcohol-soluble extract. This method was chosen because only 15 to 30 per cent of the total fats and lipoids present in tissues are obtained on exhaustive extraction with ether. The remainder is easily extracted with alcohol. This observation was first made by Hoppe Seyler[14] and was later confirmed by Erlandsen,[13] Cohn,[15] and others. As to the nature of these two fractions of lipoid material there is a diversity of opinion; thus Erlandsen believes that the ether and alcohol soluble fractions contain different lipoids, while MacLean[16] claims that the two lipoids are the same. He attempts to explain this phenomenon on the supposition that the ether fails to penetrate the tissues, the difficulty being a mechanical one. The work of Neymann and Gager, however, favors the view held by Erlandsen, since the ether and alcoholic fractions show different antigenic properties. They recommend a method for making up antigen, for which they claim superior qualities over antigens prepared by the ordinary methods. Their method is as follows: After removing as much as possible of the endocardium and pericardium, and the larger blood vessels and fat from a normal beef-heart, the muscle is ground very fine, spread in a thin layer on glass plates and dried by means of an electric

fan for twenty-four hours. The dry tissue is ground up again to a fine powder, placed in cloth bags and dried in an incubator at 37° C. for several days. The powder is then extracted with ether in a Soxhlet extraction apparatus for twelve hours, or in an ordinary wide-mouthed bottle at room temperature five or six times until the supernatant fluid is no longer colored yellow. This takes from eight to ten days. The ether-free powder is now dried and extracted with 95 per cent alcohol for ten days. It is then filtered and is ready for use.

The authors claim to have prepared ten antigens successively from ten different hearts obtaining equally good results. Their antigens have a binding power of 1 to 1600, low anticomplementary titer, and no hemolytic properties.

I have prepared three different antigens from three different beef-hearts according to the above described method and verified the claims of Neymann and Gager. The two important points established by these authors are, that the tissues used in preparation of antigens for the Wassermann test can withstand drying at room temperature; and that by first removing the ether-soluble lipoids from the tissues a better antigen is obtained. This method, however, while having the advantage over the older methods in that better antigens are obtained, yet the method of preparation is very tedious and requires from two to three weeks to prepare an antigen.

Noguchi[17] divided the extracts of normal tissue into four groups as follows:

Group I.—Insoluble in ether and hot alcohol. Consists chiefly of proteins and salts. Proteins bind complement with certain active sera; this binding is due to some other substances present in the serum not identical with the one that binds complement in the Wassermann test. Thus the presence of protein in an antigen, by its fixation of complement, would tend to obscure the Wassermann test and give false positive results with nonsyphilitic sera.

Group II.—Insoluble in ether but soluble in hot alcohol. These consist of soaps, cleavage products of proteins, and bile salts. Soaps and bile salts are strongly hemolytic and very slightly antigenic.

Group III.—Soluble in ether, alcohol and acetone. Contains saturated fatty acids, cholesterins, lipoids of unknown nature. These substances are anticomplementary or hemolytic.

Group IV.—Insoluble in acetone but soluble in ether and alcohol. These are phosphatides, among which lecithin is the best known.

Thus Noguchi prepared his well-known acetone-insoluble antigen by extracting organs with alcohol for about ten days to two weeks at 37° C. or for longer periods at room temperature. The alcohol is then evaporated at room temperature, the residue taken up with ether and precipitated with acetone.

No matter what method is used in preparing an antigen, upon one point all workers have agreed, and that is that all extractions must be done at temperatures not above 37° C. The reason for such precaution is very obvious since it was proved that the binding component of the antigen consists of "lecithin-like" substances, and certain of these substances are of a very labile nature; too long exposure to the oxygen of the air or high temperature

greatly increases the ease with which oxidation of their unsaturated fatty acids takes place. The usual method[18] for obtaining phosphatides is to get the tissues dried in the shortest possible time with a minimum exposure to air at the lowest temperature that can conveniently be employed.

In preparing substances of lipoidal nature great care was always taken to remove as much water as possible from the tissue previous to applying a solvent. This is necessary because the solubilities of the various lipoids in alcohol and ether vary with the amount of water present. There are also indications that phosphorus-containing substances other than lipoids are extracted along with the phosphatides in the presence of even minute quantities of water.[18] It was for this reason that Collison[19] suggested that solvents used for lipoid extraction should be water-free.

Noguchi improved the antigen by precipitating with acetone, i.e., by removing the acetone-soluble substances. Neymann and Gager improved it by first drying the tissue and then removing the ether-soluble substances. Taking these facts into consideration I thought that by combining the two methods, that is, by first drying the tissue, then extracting with ether, followed by an extraction with acetone, thereby removing from the tissue both the ether and acetone-soluble substances, a good antigen ought to be obtained. If in addition, the drying and extractions could be done at high temperatures, the time consumed in the process would be greatly shortened and a good antigen would be produced in a short time. It is true that most of the lipoids are thermolabile; however, this is not true of all of them. The diaminomonophosphatides, for example, contain no unsaturated fatty acids and are heat-stable. Noguchi[17] states that there seems to be a certain relationship between the number of unsaturated bonds, as determined by the iodine number, and the binding power of an antigen. The work of Neymann and Gager, however, seems to oppose this view by showing some evidence that the active principle in the antigen is a phosphatide belonging to the group of diaminomonophosphatides, which was shown by Levene[20] and others to contain no unsaturated fatty acids.

PROCEDURE

Six fresh beef-hearts were ground up in a meat grinder. A small portion was placed in ten times its weight of alcohol and kept at 37° C. to be used as a control. The remainder of the tissue was divided in two equal portions; one part was spread in thin layers over large glass plates and dried under an electric fan. It took thirty-six hours to get the tissue dry. The other portion was dried on a boiling water-bath. The dried tissues were then ground to a fine powder and each divided into four portions which were treated as follows:

1. Extracted with ether in Soxhlet for twenty-four hours.
2. Extracted with acetone.
3. Extracted with ether followed by extraction with acetone.
4. Taken up in absolute alcohol without any previous treatment.

The acetone extractions in the case of the heat-dried tissues were done in the Soxhlet extraction apparatus for twenty-four hours, while the tissues

dried at low temperature were extracted by pouring acetone over the tissues and keeping in the incubator at 37° C. with occasional shaking. These extractions continued for a week with daily changes of acetone.

All the samples after being dried were placed in absolute alcohol; the tissues prepared at low temperature were placed in the incubator at 37° C. and kept for seven days with occasional shaking. The tissues prepared at high temperatures were extracted with the alcohol on the water-bath at 70 to 75° C., just below the boiling point of alcohol, for two hours. The alcoholic extracts were then cooled and filtered through filter-paper.

All titrations were done a week later when the rest of the antigens were ready to be removed from the incubator. The proportion of tissue to alcohol was 1 gram of tissue to 10 c.c. of alcohol.

TITRATION OF ANTIGENS

One-fourth Wassermann quantities of an antisheep hemolytic system were used. The complement was taken from a pool of three or four guinea pigs and its dose was determined immediately before use. The doses used were two units of complement and two units of amboceptor.

0.25 c.c. of a 5 per cent sheep cell emulsion.
0.05 c.c. of syphilitic serum.
0.1 c.c. negative serum.

In order to obtain a syphilitic serum which just completely inhibits hemolysis, a strongly positive serum was first tested in a set of dilutions with saline, and the highest dilution which still gave complete inhibition of hemolysis was used in the test. The positive serum was inactivated for ten minutes at 56° C. The first incubation was done in the ice box for four hours, sensitized cells were then added and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1 to 10, and 1 to 40, by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it gently all the while.

A series of fifteen tubes was set up, the first four tubes receiving amounts of the 1 to 4 diluted antigen, while all the remaining tubes contained increasing amounts of the 1 to 10 dilution.

TABLE I

	BINDING POWER						ANTICOMP. PROP.					HEMOLYTIC POWER			
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1/40 ant. c.c.	.01	.02	.05	.1											
1/10 ant. c.c.					.05	.1	.2	.5	.75	1.0	1.2	.5	1.0	1.0	1.0
Final Dil.	1/4000	1/2000	1/800	1/400	1/200	1/100	1/50	1/20	1/13	1/10	1/8	1/20	1/10	1/10	1/10

The first six tubes containing antigen in dilutions ranging from 1 to 4000 to 1 to 100 each contained 0.05 c.c. positive serum, thus serving as a test for the antigenic power of the antigens. Tubes 7 to 11 inclusive contained no serum (except Tube 7) and served to demonstrate the anticomplementary property. Tube 7 contained 0.1 c.c. of negative serum, thus showing the spec-

ificity of the antigens. The last four tubes indicate the hemolytic property. Tubes 12 and 13 contained no serum and no complement. Tube 14 contained no serum and no complement and no amboceptor.

Controls were also set up with positive and negative sera without antigen to make sure that the hemolytic system was right. In writing the results the following signs were used:

++++ = complete inhibition of hemolysis
+++ = slight hemolysis
++ = half inhibition
+ = slight inhibition
0 = complete hemolysis
+? = doubtful

The following points were brought out by the experiment as seen in Table II:

1. A preliminary ether extraction improves the antigenic and anticomplementary properties of an antigen. It also removes hemolytic components by removing free fatty acids, fats, etc.

2. Subsequent extraction with acetone rather impairs an antigen by diminishing its binding power, probably by removing antigenic lipoids.

3. The outstanding feature is the fact that the phosphatides responsible for the complement fixation in the Wassermann reaction can withstand high temperatures for considerable lengths of time. Heat not only does not injure them but seems to improve them.

This experiment was repeated twice and the same results were obtained each time. Accordingly the following method was used for preparing an antigen:

METHOD FOR PREPARING AN ANTIGEN

Fresh beef-heart is ground up in an ordinary meat grinder. It is then placed in a large flask with an excess of water and autoclaved for ten minutes or just boiled for about half an hour over a free flame. The tissue is then filtered through a cloth, such as a towel, and washed a few times with hot water. This process removes free fat and extractives. The tissue is then pressed to remove as much water as possible, and dried on the water-bath or in an oven at 100° C. until completely dry. It is then ground up finely and extracted completely with ether in the following manner: The powdered tissue is placed in a filter cup such as is used in the Soxhlet apparatus for fat extraction. The cup is suspended in a flask to which a reflux condenser is attached. The cup can easily be attached to the cork by thin copper wires passing through the cork.

The advantage of this method for extracting with ether is twofold; it saves a great deal of time and ether, the whole extraction is completed in three to four hours with a small amount of ether; and second, the ether used in this manner is practically anhydrous and devoid of impurities, since only the vapors of the ether come in contact with the tissue. When the ether extraction is complete, the powder is removed from the filter cup and spread on a paper to dry. This takes but a few minutes. Twenty grams of the dry powder is then boiled with 100 c.c. of absolute alcohol on the water-bath

TABLE II

	ANTIGENIC						ANTICOMPL.					HEMOLYTIC			
TUBE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	+	++	++++	++++	++++	++++	0	0	0	+	++	++++	++++	++++	++++
A-1	++	+++	++++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
A-2	0	0	+	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
A-3	0	0	+++	++++	++++	++++	0	0	0	0	+?	++++	++++	++++	++++
B	+	+	++++	++++	++++	++++	0	0	0	+?	+	++++	++++	++++	+++
B-1	++	++++	++++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
B-2	0	0	++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
B-3	0	+	+++	++++	++++	++++	0	0	0	+?	+?	++++	++++	++++	++++
X	0	0	+?	+++	++++	++++	0	0	0	+	++	++++	++	++++	++
Y	0	0	0	++++	++++	++++	0	0	0	+?	+	++++	+++	++++	+++

A = antigen prepared at low temperature, not extracted with ether or acetone.
A-1 = low temperature, extracted with ether.
A-2 = low temp. extracted with acetone.
A-3 = low temp. extracted with ether and acetone.
B = high temperature, not extracted with ether or acetone.
B-1 = high temp. extracted with ether.
B-2 = high temp. extracted with acetone.
B-3 = high temp. extracted with ether and acetone.
X and Y are control antigens prepared by the ordinary method.

under a reflux condenser for two hours. When cool, it is filtered through filter paper and the alcoholic solution containing the antigen is placed in the ice box overnight and filtered again. The antigen is now ready for titration. It gives best results in dilutions of 1 to 10 or 1 to 20.

An antigen prepared according to the above described method was tested out on 500 sera, both positive and negative. It was checked against two antigens of proved value, one plain alcoholic, and one cholesterinized antigen. The following method was used: $\frac{1}{10}$ Wassermann quantities were employed in the tests. Three tubes were set up for each test: one tube containing 0.1 c.c. of diluted antigen and 0.02 c.c. of serum, another tube contained 0.05 c.c. of antigen, 0.02 c.c. of serum. The third tube contained no antigen and was used as a control. Two units of complement were added to all tubes and incubated in the ice box for four hours. Five per cent of sheep-cells and two units of amboceptor were then added and the next incubation was done in the water-bath at 37° C. for fifteen minutes.

The results showed a very close agreement between the three antigens. Three hundred and thirty-nine sera gave negative results with the three antigens; 122 sera gave positive results with all the antigens; 39 sera showed a discrepancy between the three antigens. Thus there was an agreement of 92 per cent and a disagreement of 8 per cent. The disagreement was with the weakly positive and doubtful sera. However, the same disagreement existed between the two control antigens. It was never found possible to obtain identical results with different antigens in weakly positive or doubtful sera.

SUMMARY

1. The beef-heart used in the preparation of antigen for the Wassermann test may be boiled or even heated in the autoclave under fifteen pounds pressure for an hour without impairing the antigen obtained by extraction with alcohol.

2. The meat may be extracted with hot alcohol without injury to the antigen.

3. A method is recommended for preparing an antigen at high temperature.

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THE CATALASE-ACTIVITY OF THE ORAL MUCOUS MEMBRANE*

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THE susceptibility to mercurial stomatitis is generally believed to vary with the healthy or unhealthy condition of the mouth, particularly of the gums. It would be desirable if the degree of this dependence could be definitely ascertained. Such a study would be likely to be more valuable if the clinical diagnosis of the oral condition could be compared with data that would not require so much personal interpretation; and especially if these data could be reduced to a quantitative basis. It occurred to us that these requirements might be met by measuring the decomposition that hydrogen peroxide undergoes in the mouth; for oral sepsis involves the presence of pus, especially about the alveolar margin, and the production of putrefactive substances, which would probably have a reducing action.

In principle, such a method would consist of taking a quantity of hydrogen peroxide into the mouth, and estimating the amount that remained undecomposed after a certain length of time, under standardized conditions. The execution of the method is as simple as its principle and no difficulty was experienced in determining the influence of various factors of technic, and in establishing a satisfactory standardized procedure.

Through the sympathetic cooperation of Dr. W. L. Wylie, we were able to try the method in the Dental Clinic of Western Reserve University. For this purpose, a schema was worked out for observing, recording, and grading the clinical conditions from the standpoint of oral sepsis. According to this schema, the conditions of the mouth were roughly graded as "normal," "bad," and "very bad." The peroxide decomposition or "catalase index," as it may be called, was then determined. When twenty-five cases had been collected, the two values were compared. This showed that nine-elevenths of the mouths classed clinically as "normal" gave indices below a certain level; while two-thirds of the "very bad" mouths had indices materially above this level. The indices of the "bad" mouths did not exceed those of the "normal" mouths. It appears, therefore, that the catalase index reflects "very bad" clinical conditions of the mouth, but does not indicate lesions that are clinically quite evidently "bad." Even more disturbing is the fact that a few perfectly normal mouths gave a "very bad" index; and a very bad mouth gave a "normal" index. It must be recognized, of course, that there need not always be parallelism between the morphologic changes and the chemical changes that result from inflammatory processes; and the possibility of parallelism between the catalase index and the liability to mercurial stomatitis should therefore not be rejected without a trial, which has

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not yet been made. However, the results suggested that the condition of the teeth and gums is not the only, and perhaps not even the main factor to influence the catalase index of the mouth. This is confirmed by the observation that if a number of peroxide washings of the mouth are made in quick succession, the rate of decomposition remains constant, after the second washing; i.e., after the detachable oxidizable material has been removed by the first washing, the catalase action is a fixed property of the mucous membrane itself.

To throw further light on the question of the fixed catalase activity it was planned to follow the catalase index of "normal" individuals over a period of several weeks. Since their dental condition would naturally remain approximately constant during this time, the index should also remain approximately constant if it depends mainly on the dental condition. Considerable variations, on the other hand, would indicate the importance of

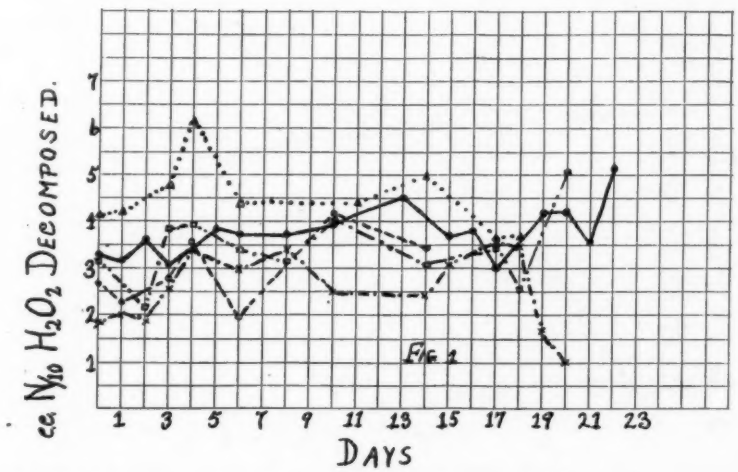

Fig. 1.—Normal course of catalase index over longer periods.

. ———— O - - - - - - - - △ ..... X .—.—.—.—. □ ..—..—..—.. Indicate the curves (first peroxide treatment) for five different individuals.

nondental conditions. These tests were carried out on five persons. Fig. 1 shows that the figures for each subject ran, on the whole, fairly uniformly but marked rise or depression of the index occurred occasionally with most of the subjects. These changes may be of short or of fairly long duration.

It appears, therefore, that conditions other than dental do have a considerable influence. A few attempts were made to find such conditions; but increase of salivation by chewing paraffin, suppression of salivation by atropine, and the intercurrence of "sore throat" did not appear to have any definite effect on the index. Perhaps digestive and febrile conditions, which would modify the "coating" of the tongue, may be concerned. This constitutes a subject for future investigations. The establishment of a method for the determination of the catalase index of the mouth may perhaps also help

to solve broader questions as to the significance of the catalase activity of mucous membranes.

Method of Determining the Catalase Index of the Mouth

The following technic was fixed upon after the various factors and conditions entering into it had been investigated (as will be described in the appendix). It involves a preliminary rinsing of the mouth with 1 per cent sodium bicarbonate solution and with two portions of water, to remove mucus and detritus; then a definite quantity of titrated hydrogen peroxide solution is taken into the mouth and forced between the teeth. After one minute, the peroxide is washed from the mouth. The whole procedure is then repeated, so that two treatments with peroxide are given and two sep-

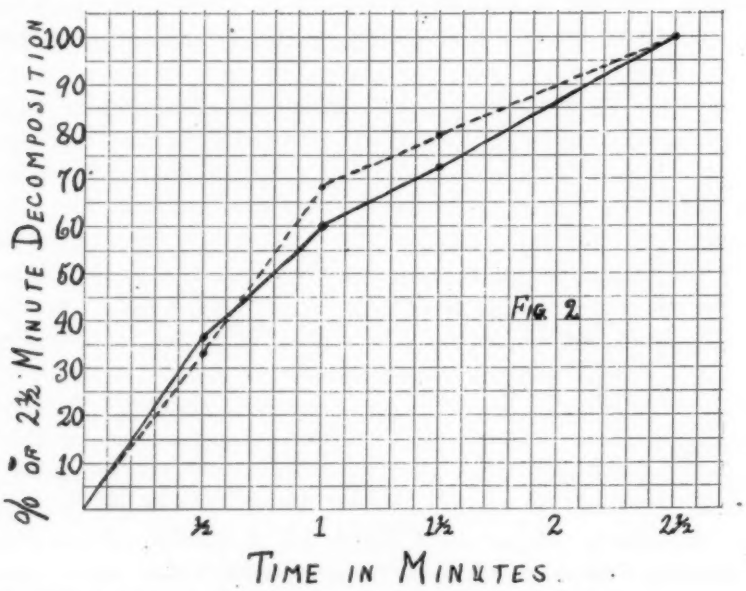

Fig. 2.—Time of sojourn of peroxide in mouth on catalase index.

————First peroxide treatment; 100=5.40 c.c. of N/10 H_2O_2 consumed.
- - - - - - - - Second peroxide treatment in close succession after a first treatment of the same duration; 100=4.28 c.c. of N/10 H_2O_2 consumed.

arate samples collected, and in each of these the undecomposed peroxide content is determined, at once. The first sample would contain more of any detachable material, leucocytes, etc., and may, therefore, be the better index of the presence of pus, while the second sample would reflect more definitely the catalase activity of the mucous membrane proper, and may, therefore, prove to give more important data; but until this point has been decided it appears desirable to make both determinations. In general, while the decomposition is always greater in the first than in the second sample, the two sets of figures run fairly parallel. If the samples are allowed to stand, the decomposition of the peroxide continues, and we have therefore also titrated all our specimens at the end of twenty-four hours; but the results paralleled in their general direction those of the immediate titration, except

that they were rather more irregular, presumably because of the slow decomposition of variable amounts of detritus. It would therefore be superfluous to reproduce our data and we would not consider the twenty-four-hour titration as worth the trouble. This reduces the details of the method to the following

STANDARDIZED TECHNIC

1. The patient is given 30 c.c. of a 1 per cent $NaHCO_3$ solution and instructed to take the entire quantity into the mouth and to rinse thoroughly both the mucosa and the teeth. The washing is discarded.

2. Next 30 c.c. of distilled water are given to the patient, with directions to wash mouth and teeth even more thoroughly than with the bicarbonate. This washing is discarded.

3. Another portion of distilled water is used, and No. 2 is repeated.

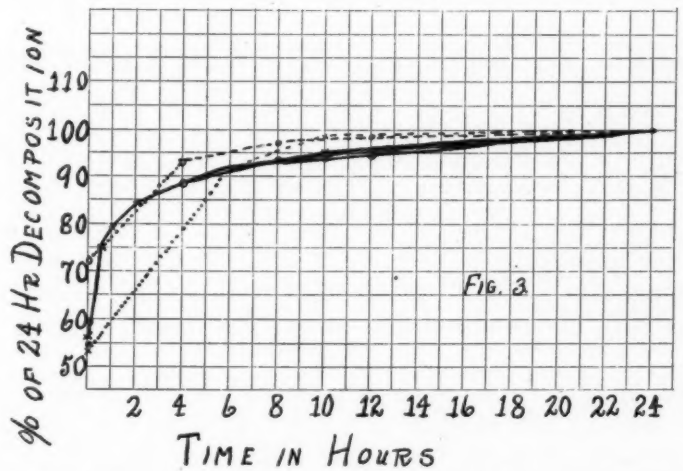

Fig. 3.—Deterioration curve of peroxide washings in vitro at room temperature.

————First peroxide treatment (three subjects).
- - - - - - - - Second peroxide treatment (two subjects).
The curve from the first peroxide treatment between zero and four-hour period is derived from a separate experiment upon one of the subjects.
The dotted lines (.....) in the second peroxide treatment indicate that the form of the curve in that region has not been determined.
The absolute values for c.c. N/10 peroxide decomposed in 24 and 48 hours were:

Subject	Washing	c.c. Decomposed in 24 hours	c.c. Decomposed in 48 hours
12	first	3.69	3.79
H	first	3.34	3.25
H	second	1.98	2.00
11	first	3.25	3.43
11	second	1.95	2.02

4. Five c.c. of a titrated U.S.P. solution of H_2O_2 are measured carefully with a pipette and diluted with distilled water to 20 c.c. The patient is instructed to take the entire quantity into the mouth, draining the glass; then to close the teeth and force the peroxide mixture through the teeth, first front, then right side, then left side, etc., repeating this procedure for the duration of one minute. This process also washes the mucosa of the mouth. The patient is warned against getting the fluid so far back in the mouth that there will be danger of swallowing some of the peroxide; and

against forcing the fluid through the teeth so violently that some might escape through the lips.

At the end of one minute, the patient is given a short wide-mouthed bottle (graduated at 100 c.c.) and directed carefully to allow the fluid in the mouth to flow into it. All foam in the mouth should be transferred to the bottle. The patient is again warned against swallowing until the procedure is completed.

5. Thirty c.c. of distilled water are introduced into the mouth and both mucosa and teeth rinsed well (as in No. 2) except that the washing is not discarded but added to that obtained in No. 4.

6. The procedure outlined in No. 5 is repeated.

7. A second sample of H_2O_2 is then given to the patient, repeating the procedures, 4, 5, and 6, and collecting in a separate bottle. This also serves as a check; indicating whether or not the patient has lost any of the first sample; for the second must always show definitely less decomposition than the first.

8. As soon as both samples have been collected, distilled water is added to both bottles so as to dilute the contents to 100 c.c. The solutions are well mixed. When the foam has risen to the top and the watery layer is only slightly cloudy (a few minutes) 10 c.c. are removed and added to 150 c.c. of water in a beaker. Twenty-five c.c. of 25 per cent H_2SO_4 are then introduced (an equivalent amount of the concentrated acid generates too much heat), and the undecomposed peroxide is titrated with tenth-normal $KMNO_4$. The "permanganate number" so obtained is subtracted from the titration value of the H_2O_2 before introducing it into the mouth. This is determined as follows:

Five c.c. H_2O_2 are diluted to 100 c.c. with distilled water and of this 10 c.c. are removed and titrated in exactly the same manner as the mouth washings. This figure is the theoretical value. The theoretical value minus any given titration equals the cubic centimeters of tenth-normal $KMNO_4$ equivalent to the cubic centimeters of tenth-normal H_2O_2 decomposed.

All data are then recorded in figures representing cubic centimeters of tenth-normal H_2O_2 decomposed.

CONCLUSIONS

1. An index of the catalase activity of the oral mucous membrane can be obtained by determining the rate of decomposition of hydrogen peroxide under standardized conditions.

2. The catalase index may be defined as the amount (cubic centimeters) of tenth-normal hydrogen peroxide decomposed by one minute's contact with the mucous membrane. Two successive determinations are made, of which the first is somewhat higher because it includes the detachable detritus.

3. The index for the first determination lies between 1.35 and 8.20 c.c.; of the second determination between 0.64 and 5.50 c.c.; but unless the oral condition is very bad, it rarely exceeds 4 c.c. for the first, and 3 c.c. for the second determination; and the median value is 3.3 c.c. for the first and 2.29 c.c. for the second determination.

4. Clinical conditions of the mouth classed as "very bad" give values generally above 4 c.c. for the first and 3 c.c. for the second determination; with the median value of 6.0 for the first and 5.0 for the second determination.

5. The catalase index of a given individual varies somewhat from day to day, for reasons that are not yet explained.

6. It is not affected by increasing or decreasing the flow of saliva (chewing gum, atropine), by smoking, or by "sore throat."

APPENDIX: EXPERIMENTAL DATA

The Influence of Modifications of Technic.—In selecting the standardized technic, various modifications were tried, as to the treatment of the mouth; and as to the rapidity of the decomposition of peroxide in vitro and in the mouth. These are important as justifying the selections of the method, and some have an interest of their own.

The Importance of the Preliminary Rinsing of the Mouth.—When the mouth was washed with bicarbonate solution and with one rinsing of water, and peroxide added to the mixed washings, about 1 c.c. of tenth-normal H_2O_2 was decomposed; but if the mouth was again rinsed with water, the latter solution did not decompose any peroxide. Accordingly, the standard procedure removed all the loose decomposable material.

Preliminary Brushing of the Teeth.—Ordinarily, the indices were determined at least two hours after the last meal, without brushing the teeth. It was found that the brushing did not influence the index materially, at least for persons with ordinarily hygienic mouths. The average indices of three series of experiments were practically the same when taken ten minutes after brushing the teeth; when two hours had elapsed; and when a meal and a longer period had intervened since the brushing.

The Time that Had Elapsed Since the Last Meal.—This was also found to be unimportant; for the index taken ten minutes after a meal may be either a little more or a little less than that taken several hours after a meal; the teeth not being brushed in either case.

The Time of Contact of the Peroxide with the Mouth.—This is a very important factor as shown by Table I and Fig. 2.

TABLE I

CUBIC CENTIMETERS OF TENTH-NORMAL H_2O_2 DECOMPOSED WHEN KEPT IN THE MOUTH FOR VARIOUS PERIODS OF TIME

	FIRST SAMPLE		SECOND SAMPLE	
Determined	At Once	After 24 Hours	At Once	After 24 Hours
Time ½ min.	2.00	3.34	1.44	1.98
Time 1 min.	3.22	4.87	2.90	4.20
Time 1½ min.	3.93	5.21	3.40	4.65
Time 2½ min.	5.40	6.21	4.28	5.85

It is seen that the decomposition increases materially with the time of contact, and the form of the curve, as well as general considerations, indicate that it will do so indefinitely. An arbitrarily fixed period must, there-

fore, be selected for comparative experiments. One minute was chosen because this lies beyond the steepest part of the curve and therefore beyond the region of maximal error; and on the other hand, it avoids the discomfort of too excessive foam and of the disagreeable taste that may develop if the contact is further prolonged.

Effective Contact of Peroxide with Teeth.—The standard directions for drawing the peroxide through the teeth aim to secure thorough contact. This is doubtless a wise instruction, but it need not be feared that the results will be altered if it is only negligently carried out; for comparative experiments gave practically the same results if the solution was only slightly agitated by movements of the cheeks and of the tongue.

Number of Rinsings Necessary to Obtain All the Peroxide from Mouth.—This was fixed at two, since the third washings were free from titrable quantities of peroxide.

The Catalase Index of Successive Peroxide Treatments.—When the catalase index is determined several times in sequence, on the same person, it is found that the index of the first sample is always materially higher than that of the second; but from the second sample on, the index remains constant (Table II). This shows that the catalase action is produced by the living mucous membrane. It also establishes the fact that it is not necessary to make more than two peroxide treatments to obtain the catalase index of the mucosa.

TABLE II

CATALASE INDEX OF SUCCESSIVE PEROXIDE TREATMENTS

(Cubic Centimeters of Tenth-normal H_2O_2 Decomposed After One-half Minute Contact)

SUBJECTS	A.R.	D.	W.J.C.
Peroxide Treatment 1:	2.59	2.07	1.86
Peroxide Treatment 2:	2.04	1.51	1.46
Peroxide Treatment 3:	1.90	1.49	1.23
Peroxide Treatment 4:	1.88	1.34	1.21
Peroxide Treatment 5:	1.85	1.33	1.30
Peroxide Treatment 6:	1.89	–	1.30

The Time-Curve of the Catalase Index.—This was studied by allowing the peroxide washings to stand at room temperature in a glass-stoppered bottle, and repeating the titration at intervals. Control tests on an equivalent dilution of peroxide with distilled water showed that this undergoes no change in twenty-four hours, and very little loss in forty-eight hours; after this time there is apt to be some deterioration. The results of the mouth treatments are shown in Fig. 3. In this the curves are drawn to the scale of 100 — the cubic centimeters of tenth-normal H_2O_2 decomposed in twenty-four hours, for each subject. The H_2O_2 was kept in the mouth for one-half minute. It is seen that the curves run practically parallel, for both the first and the second specimens. They have the parabolic form; so that if the decomposition at the end of twenty-four hours is taken as 100, about 58 per cent of this is decomposed within one-half minute; 85 per cent in two hours; 88 per cent in four hours; 92 per cent in eight hours; and 111 per cent in forty-eight hours.

The Daily Variations in the Catalase Index.—Repeated determinations during two or three weeks were made on five subjects with fairly normal mouths. The catalase indices were recorded only for the first washings with peroxide—one minute contact. The curves drawn from the immediate titrations are shown in Fig. 1. Their significance has already been discussed.

The Influence of Experimental Conditions—Salivation.—This was tried in two cases, by taking the one-minute contact index after chewing gum and smelling strong acetic acid. The results of two determinations are a trifle lower than the normal average of each individual, but they are well within the normal variation. This is shown in Table III.

TABLE III

THE INFLUENCE OF SALIVATION ON CATALASE INDEX

SUBJECTS:	R.L.H.	H.P.C.	AVERAGE
First Sample Controls	3.76	2.96	3.36
Increased saliva flow	3.61	1.70	2.65
SUBJECTS:	R.L.H.	H.P.C.	AVERAGE
Second Sample Controls	3.12	1.48	2.30
Increased saliva flow	3.13	1.05	2.09

Suppression of Saliva by Atropine.—This was tried on three subjects each with one experiment, one minute contact. The results (Table IV) show that there is no constant effect, but only the normal variations.

TABLE IV

SUPPRESSION OF SALIVA BY ATROPINE ON THE CATALASE INDEX

SUBJECTS	R.L.H.	H.P.C.	A.R.	AVERAGE
First Sample Control	3.76	2.96	3.35	3.35
Decreased saliva flow	5.18	1.00	5.09	3.75
SUBJECTS	R.L.H.	H.P.C.	A.R.	AVERAGE
Second Sample Control	3.12	1.48	2.49	2.36
Decreased saliva flow	3.55	0.68	3.53	2.11

The Effect of Smoking.—The values shown in Table V were obtained by smoking a cigarette for ten minutes between two successive determinations; and the control experiments by permitting the same period of time to lapse without smoking. The indices (one minute contact) run somewhat lower for the smoking, but within the normal variations, so that the difference may be accidental.

TABLE V

THE INFLUENCE OF SMOKING ON THE CATALASE INDEX

SUBJECT	R.L.H.	H.P.C.	O.W.B.	A.R.	AVERAGE
First Sample Control	4.26	3.61	3.69	3.55	3.79
Second Sample Control	3.12	1.48	2.09	2.49	2.29
First Sample (Smoking between samples)	4.26	3.55	3.71	2.67	3.54
Second Sample (Smoking between samples)	2.75	1.87	1.68	2.20	2.12

"Sore Throat."—One of the subjects, who was under observation for three weeks, to determine the variability of the normal index, developed a

cold and "sore throat," sufficiently severe to confine him to bed; the catalase index, however, ran a practically steady, normal course; so that in this case, at least, the pharyngitis did not affect the catalase index.

The Range of the Catalase Index in Normal and Pathologic Conditions of the Mouth.—The data for this study were secured from the Dental Clinic of Western Reserve University through the kind collaboration of W. L. Wylie.

A careful clinical examination was made before the chemical tests; recording especially such conditions as might indicate oral sepsis: The odor of the breath (which was found a very useful criterion); caries; the condition of the gums, especially recessions and pyorrhea; the condition of the mucosa of the various portions of the mouth; and the presence of any definite lesions, patches or ulcers. According to this examination, the patients were grouped as

N = *Normal,* i.e., without definite inflammatory conditions;
B = *Bad,* with definite active caries, receded, or inflamed gums, or patches; and
BB = *Very bad,* with marked inflammatory conditions, and especially pyorrhea or other distinctly pathologic lesions.

The indices of the first and second tests are shown in Table VI, arranged in the order of the magnitude of the index of the first test. The fourth column gives the clinical grouping.

TABLE VI

CATALASE INDEX (1 MINUTE CONTACT) OF NORMAL AND PATHOLOGIC MOUTHS

CASE NUMBER	FIRST TEST INDEX CATALASE	SECOND TEST INDEX CATALASE	OF MOUTH* CONDITION CLINICAL
20	1.35	1.35	B
25	1.36	–	B
16	1.81	1.15	N
22	1.82	1.27	*N
26	2.04	–	B
6	2.15	–	N
24	2.35	–	B
30	2.50	0.64	BB
5	2.96	–	N
2	2.96	–	N
3	3.35	–	N
18	3.39	2.11	N
21	3.41	2.27	N
23	3.44	–	B
14	3.67	2.14	BB
1a	3.76	2.44	N
29	3.88	2.19	B
17	4.16	1.74	B
4	4.33	–	N
27	4.53	4.88	B
19	5.51	4.60	N
15	5.88	5.50	BB
13	5.95	5.05	BB
31	6.69	5.04	BB
28	8.20	4.71	BB

*N—"Normal."
B—"Bad."
BB—"Very bad."

Table VII gives the range medians for the clinical groupings.

TABLE VII

THE CATALASE INDEX OF THE CLINICAL GROUPS

GROUP	CATALASE INDEX OF FIRST TEST:			CATALASE INDEX OF SECOND TEST:		
	No. of Cases:	Range:	Median:	No of Cases:	Range:	Median:
Normal	11	1.81-5.51	3.23	7	1.15-4.60	2.27
Bad	8	1.35-4.53	3.44	4	1.35-4.88	1.96
N plus B	19	1.35-5.51	3.33	11	1.15-4.88	2.19
Very bad	6	2.50-8.20	5.95	6	0.64-5.50	4.87

It is evident that the "normal" and "bad" cases must be grouped together as "normal," so far as the catalase index is concerned. A fairly definite dividing line between these and the "very bad" cases lies at 4.0 for the first washing and 3.0 for the second washing; of the "normal to bad" cases, three-fourths (i.e., fifteen-nineteenths) lie below 4.0 for the first washing and nine-elevenths lie below 3.0 for the second washing; whereas of the "very bad" cases, two-thirds (i.e., four-sixths) lie above this dividing line, both for the first and for the second washing.

THE POTENTIATION OF NOVOCAINE SOLUTIONS*

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IN the production of novocaine the efforts of workers in the field of synthetic drugs have been rewarded with decided success. For several years this drug has most nearly fulfilled all the requirements of the ideal local anesthetic, almost entirely superseding other substances. While conceded to be somewhat less powerful in anesthetic action than cocaine, it has gained a reputation for efficiency which, when considered with its high solubility, stability in solution even when sterilized by boiling, its low toxicity and freedom from local irritating effects, has placed it at the top of the list in point of general applicability. One of the most important advantages of novocaine is the possibility of safely injecting large quantities of the solutions. This fact has greatly broadened the scope of local anesthesia and has been largely responsible for the development of regional anesthesia upon its present scientific basis.

While novocaine is the least toxic of local anesthetic substances, it must be remembered that all such drugs are protoplasmic poisons with greater or less affinity for nerve cells. The toxicity of novocaine is not an entirely negligible factor. When local infiltration was limited to minor operations the injection of small quantities of weak solutions kept the method well within

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the limits of safety. But with the introduction of regional and nerve block methods the total quantity and concentrations of the solutions have increased. In certain quarters deaths have been reported and the local anesthetic held responsible. These deaths have not only been those in which large amounts of solutions have been employed. Fatalities have followed the injection of small amounts of solution in cervical plexus block, and in sacral anesthesia.[24] Of a total of forty-three deaths investigated by a special committee of the American Medical Association,[14] twenty occurred during the performance of tonsillectomy. It is gratifying to observe, however, that of the total number of deaths novocaine was employed in only two cases, although it enjoyed a far wider clinical use than any other drug.

While the safety of major operations rendered anesthetic by the use of large quantities of novocaine solutions properly employed has been repeatedly demonstrated, the sphere of usefulness of the method would still be farther extended by the introduction of a drug even less toxic than novocaine. It is reasonable to expect that synthetic chemists will produce a local anesthetic drug having a greater margin of safety than novocaine. Many such intended preparations have been the subject of pharmacologic investigation. At the present time study of upwards of thirty new preparations is being carried out under McGuigan's[15] directions at the University of Illinois. Until the superiority of some new preparation has been demonstrated, however, the only means of improvement consists in the possible potentiation and prolongation of the anesthetic action of those drugs now in use.

Various methods have been proposed for the potentiation of novocaine solutions. For all of these it is claimed either that the anesthetic power of the novocaine is increased to such an extent that satisfactory anesthesia may be obtained with weaker solutions, or that the duration of the period of anesthesia is prolonged. The only method of universally acknowledged benefit has been the addition of adrenalin. To Braun[2] is largely due the credit of first introducing and perfecting the use of this agent in local anesthesia. There is probably no other single factor which has more encouraged the use of local anesthesia. By means of the vasoconstriction of adrenalin the anesthetic solution is retained within the tissues longer, which both intensifies and prolongs the effect for a period of time usually sufficient for the performance of most major operations.

Sodium bicarbonate has been used as an adjuvant to novocaine. Gros[6] determined experimentally that the bases of local anesthetics have a more intense action than their salts for the reason that they act more quickly and in weaker solutions than their salts. At first he added the amount of sodium bicarbonate calculated to bind the chloride, later finding that an excess of alkali gave still greater efficiency so that isotonic solutions of sodium bicarbonate or phosphate were used. He maintains that a novocaine-bicarbonate solution is five times as active as an equimolar novocaine hydrochloride solution. This is explained by the liberation of the free bases which penetrate the nervous tissues more readily than the water soluble salts. The anesthetic potential of novocaine salts was thus thought to depend upon the degree to which the free base was dissociated. Läwen[13] made use of this

principle by adding sodium bicarbonate to his formula for sacral anesthesia, which is still in general use in Germany. Sollman[22] confirmed Gros's results in experiments upon frogs and rabbits but in applying it to the human skin he found very disappointing results, the alkaline solutions being less anesthetic than the same solutions without the alkali. Gros and Läwen describe favorable effects both experimentally and clinically. On the other hand Allen[1] and Labot[12] caution against the danger of adding alkali to the anesthetic solution. They even advise against sterilizing of the instruments in an alkaline solution as being a source of possible contamination which is destructive to the anesthetic agent. Others maintain that the alkali splits the adrenalin. Braun found the addition of alkali to be inferior in duration, intensity and diffusive power to the ordinary method of preparation which gives a solution nearly neutral in reaction. Meeker and Scholl[16] were unable to observe any benefit in the addition of sodium bicarbonate to the formula for sacral anesthesia.

Potassium sulphate was first used as a means of potentiation by Hoffmann and Kochmann.[10] Their experimental work included both the sciatic nerve of the frog and the intracutaneous test on man. By the former method a potentiation of two and two-thirds times was observed and by the latter five times in terms of dilution. They recommend the addition of 0.4 to 2 per cent potassium sulphate to the novocaine solution. As the result of experimental work Sollman concluded that mixtures of the anesthetic with potassium sulphate give only simple summation and not potentiation in terminal infiltration anesthesia. After a wide clinical experience Farr[4] has been unable to satisfy himself that with the exception of adrenalin any of the drugs recommended for potentiation possess any considerable advantage.

Calcium chloride has been employed to improve the anesthetic solution. Harris[8] holds that this chemical greatly prolongs the anesthesia probably by delayed absorption of the solution. He adds from 0.25 to 1 per cent depending upon the length of time it is desired the anesthesia should last. He also recommends the addition of both calcium chloride and potassium sulphate. In spite of these claims for the efficiency of calcium chloride it has never found favor with other surgeons.

Sodium sulphate was added by Strauss[23] to improve his formula for sacral anesthesia. The advantage he claimed was that it prevented the decomposition of adrenalin, thereby prolonging anesthetic action.

Chloretone has also been used as an adjuvant. Harris[8] adds 1 per cent chloretone (chlorbutanol) as sterile crystals after the solution has cooled. It is soluble only to the extent of eight parts to a thousand, so that the addition of 1 per cent simply insures a saturated solution, and the remaining few undissolved crystals settle at the bottom as a slight harmless deposit. Chloretone is itself a powerful local anesthetic also a hypnotic and antiseptic agent. Injected hypodermically into the tissues it is quite irritating but is followed by marked anesthesia, the site of injection remaining later as a painful induration for some time. According to Harris it intensifies the action of novocaine although it seems never to have been employed by other surgeons for this purpose.

Quinine urea hydrochloride and novocaine do not synergize. But it has been suggested that the addition of quinine urea hydrochloride to the novocaine solution may prolong the anesthesia. The long duration of the anesthesia resulting from the injection of quinine salts alone in strengths of 1 per cent and greater is due to an exudate of fibrin.[9] Anesthesia may last from several hours to four or five days. It is questionable whether the prolonged anesthesia is sufficient compensation for the risk of delayed union and the possibility of an occasional slough. But the addition of a small amount of the drug, 0.1 to 0.2 per cent, to the novocaine solution to prolong the anesthesia might prove a decided advantage, as for example in block of the sacral nerves in obstetrics.

Oils and Gelatine.—Prolongation of anesthetic action has been attempted by dissolving the novocaine in a medium of oil. This has been found upon practical tests to be unsatisfactory and to possess many disadvantages. Oils are absorbed only by the lymphatics which act more slowly. This prolonged retention in the tissues should prolong the local effect and permit it to be almost entirely exhausted locally. But such solutions are entirely impractical due to the poor diffusion of oily substances through the tissues and consequent weak anesthetic action. They may also act as local irritants.

Muroya[18] has shown in experiments upon rabbits that 5 per cent gelatin in the solution delays its absorption and increases its efficiency. Allen employed gelatine in sacral injections but was uncertain of any advantages. Braun has failed to notice any increase in local effect from cocaine by the addition of gelatine. He sterilized the solutions carefully which according to Klapp[11] so alters the gelatine that its property of delaying absorption is lost.

Magnesium salts have been given considerable prominence through the writings of Meltzer.[17] The intracerebral injection of magnesium sulphate induces a state of general inhibition. Subcutaneous and intravenous injections produce deep narcosis and complete muscular relaxation. The application of magnesium sulphate to nerve trunks has been shown to block conductivity and abolish excitability. Spinal anesthesia has been produced and painless operations performed below the site of injection. This salt has also been employed as an adjuvant in general narcosis and for its alleged synergistic action with morphine. No record is available of its effect when added to a local anesthetic solution so that the possibility of potentiation remains to be investigated.

METHODS

The comparative anesthetic activity and toxicity of local anesthetic drugs has been investigated experimentally ever since substitutes were proposed for cocaine. The possibility of potentiation of these drugs has received but very little experimental investigation. The large mass of conflicting clinical data which has accumulated is of little help in understanding the value of the various mixtures. In view of the great practical importance of the subject, it has seemed worth while to carry out the following investigation. The current experimental methods of determining anesthetic potential have

been followed closely in determining the values of the potentiation formulas. Among laboratory tests that have been devised for this purpose there are six, each of which appears to have a bearing upon the clinical application; (1) Block of the sciatic nerve of the frog by direct application to the nerve trunk; (2) Paralysis of sensory nerve fibers of the frog's sciatic by direct application of the lumbosacral plexus; (3) Surface anesthesia of the frog's skin by immersion; (4) Anesthesia of the rabbit's cornea by irrigation of the conjunctival sac; (5) Mucous membrane anesthesia of the human tongue by immersion; (6) Cutaneous anesthesia in the dog and man by dermal wheals produced by intracutaneous infiltration.

I. BLOCK OF THE SCIATIC NERVE OF THE FROG

Paralysis of the motor fibers by direct application to the sciatic nerve of the frog is a method very commonly used for testing local anesthetic action. It is simple in application and outside factors modifying anesthetic effect are reduced to a minimum. For this reason it would appear that results should be more exact.

Method.—The neuromuscular preparations were made so as to include the lower end of the leg from the knee down, and the entire sciatic nerve from the knee to where it enters the pelvic girdle. The nerve was carefully isolated by blunt scissors dissection without traction or other trauma to the nerve trunk. Preparations were used as soon as made, always being tested beforehand and found active.

In applying the test the method described by Sollman was followed. The nerve trunk was immersed in the solution contained in a small trough cut into a block of paraffin. The excitability was tested with the platinum electrode of an induction coil, the usual apparatus for stimulation in laboratory experimentation. The same strength of current was used for stimulation of all preparations, and was the least that could be detected when the electrodes were placed on the tip of the tongue. The stimuli were applied one centimeter from the cut end of the nerve. Paralysis seems always to travel from

TABLE I

TIME REQUIRED FOR BLOCK AND RECOVERY OF THE SCIATIC NERVE*

SUBSTANCES	8%	4%	2%	1%	½%	¼%	⅛%	1/16%	
Magnesium sulphate	0	0	0						
Gelatine		0	0	0					
Sodium sulphate	50+30	0							
Calcium chloride	58+34	0	0						
Sodium bicarbonate	45+36	0	0						
Sodium chloride	33+34	0	0						
Chloretone				10+22	15+19	43+18	0	0	
Potassium sulphate	11+21	13+21	18+23	37+35	43+28	68+24	90+22	0	
Potassium chloride	7+14	16+13	20+18	27+16	42+12	59+22	86+29	0	
Potassium bicarbonate	12+13	14+12	24+17	25+14	39+26	73+21	73+30	0	
Quinine urea HCl.	25+80	31+48	50+30	77+30	90+30	0	0		
Novocaine	11+6	15+22	21+24	27+23	37+16	64+15	78+22	0	
Cocaine	11+15	19+25	29+17	35+24	37+24	75+24	0	0	
Distilled water									40+32
Desiccation									16+4

*The number to the left of the + indicates the number of minutes required for the production of nerve block. The number to the right of the + denotes the minutes required for recovery. The same system is followed in other tables.

the cut end of the nerve toward the muscle so that when the block was complete in the distal segment of the nerve a response could still be elicited by moving the electrodes toward the muscle. This probably indicates difficulty in penetration of the nerve sheath proper, and more rapid diffusion from the cut end of the nerve trunk between fasciculi. The same feature was observed, however, when the cut end was tied with fine thread and when only an intermediate segment of the nerve trunk was immersed.

Preparations were tested at five-minute intervals by grasping the tip of the nerve trunk with a fine iris forceps, lifting it out of the trough and applying the electrode at a point a centimeter from the end. The complex and troublesome pieces of apparatus described by Läwen and Clossen[3] for stimulation without handling the nerve trunk are unnecessary, especially when one considers the great limitations of the method itself, as will later be pointed out.

The end point was considered reached when no muscular contraction whatever could be elicited. Irritability does not cease suddenly and the criteria of paralysis have probably been applied differently by different investigators. After complete paralysis the preparations were kept in physiologic salt solution and tested at five- to ten-minute intervals for the return of function.

All experiments were conducted at room temperature. The ordinary Leopard frog (Rana pipens) was used. Substances to be tested were dissolved in physiologic salt solution (0.9 NaCl.) unless otherwise stated. The concentration of the drugs was in arithmetical progression, 8, 4, 2, 1, ½, ¼, ⅛, 1/16, and 1/32 per cent respectively.

Results.—In order to distinguish simple summation of anesthetic actions from true potentiation it is first necessary to determine the action of each constituent of the mixtures separately. For all practical purposes if complete block does not occur within an hour it had best be disregarded. Table I expresses the comparative actions of these substances. Each result represents the average of several trials. Cocaine has also been included for comparison. It is shown that cocaine, novocaine and potassium salts are approximately equally anesthetic. The small variations might well be within the limits of experimental error. Chloretone in saturated solution (0.8 per cent) and ½ per cent strength is twice as powerful as cocaine and novocaine, while quinine urea hydrochloride is inferior. The action of the inert salts in 8 per cent concentration is not to be regarded as an anesthetic action, but more the effect of any highly concentrated salt solution with no anesthetic power, although C. P. sodium chloride blocked in thirty-three minutes while magnesium sulphate was ineffective after an hour. A hypotonic effect is seen in the action of distilled water which blocked within forty minutes. Desiccation, of course, produces prompt nerve block which must be guarded against especially in the small segment of the nerve crossing from the paraffin trough to the muscle.

The potentiation values of the mixtures is best measured by mixing equal parts of novocaine and the solution to be tested for potentiation. Minimal effective doses as used by Sollman are very difficult to determine and

must be correspondingly inaccurate. The velocity index, or speed of paralysis, is more accurate than the index of dilution or minimal effective concentration in addition to being an easier and simpler method.

Table II expresses the results of the potentiation tests. Calcium chloride, magnesium sulphate, sodium sulphate and gelatine were all chemically inert alone and likewise showed no potentiation whatever. These values are approximately the same as those of novocaine alone. Mixtures of quinine urea hydrochloride are less active than the same strength of novocaine alone. Investigators have consistently shown that there is no gain in anesthetic power by the addition of other local anesthetic drugs to the novocaine solution. The novocaine-chloretone values represent simple summation without potentiation: that is, the combined action of the ¼ per cent strengths block in the average of their half per cent strengths acting alone. When potentiation has taken place the velocity of action should be shorter than the average of

TABLE II

ACTIVITY OF POTENTIATION MIXTURES

MIXTURES	½%	¼%	⅛%	1/16%	1/32%	
Novocaine and calcium chloride	35+25					
Novocaine and magnesium sulphate	36+12					
Novocaine and sodium sulphate	34+22					
Novocaine and gelatine	39+18					
Novocaine and quinine urea HCl.	46+21					
Novocaine and chloretone		18+15	37+21	0		
Novocaine and potassium sulphate	13+21	27+19	50+21	0		
Novocaine and potassium chloride	12+20	29+18	34+20	40+24	0	
Novocaine and potassium bicarbonate	5+18	9+11	14+13	30+23	63+32	0
Novocaine and sodium bicarbonate	7+21	13+18	16+14	30+22	49+15	0

TABLE III

ANESTHETIC ACTIVITY OF NOVOCAINE AND HALF PER CENT ALKALI IN DISTILLED WATER

NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%	1/256%
Sodium bicarbonate ½%	7+13	9+20	10+20	14+16	22+15	37+20	50+27	0
Potassium bicarbonate ½%	5+21	6+18	8+12	11+12	19+17	15+17	23+27	39+22

TABLE IV

COMPARATIVE ACTIVITY OF NOVOCAINE AND HALF PER CENT SODIUM BICARBONATE IN DISTILLED WATER AND IN PHYSIOLOGIC (0.9 PER CENT) SALT SOLUTION

½ SODIUM BICARBONATE AND NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%
Physiologic salt solution	7+21	13+18	16+14	30+22	40+15	0	
Distilled water	7+13	9+20	10+20	14+16	22+15	37+20	50+27

the next higher dilution. Novocaine and potassium sulphate have a combined action in ½ per cent solution of 13 + 21, which approximates the velocity of the 4 per cent strengths working alone. The average of the next higher dilution of each acting separately (37 + 35 and 27 + 23) is 32 + 29. The combined action of the two therefore gives a velocity index higher than the average of the sum of their separate actions, which is an example of true synergism or potentiation. The extent of potentiation is roughly two or three times. A similar result is seen in the combined action of novocaine and potassium chloride. The action in combination with the alkalis was still

further intensified, approximately four or five times. These results compare favorably with those of Gros and Sollman. Gros at first added just sufficient alkali to bind the hydrochloride. He found later that an excess of the bichloride was still more efficient and then employed isotonic bicarbonate solution. We tested this principle by combining higher dilutions of novocaine with a constant concentration of alkali (½ per cent). Since the constant content of the bicarbonate remained nearly isotonic, distilled water was employed as the solvent instead of physiologic salt solution. These results expressed in Table III are the highest values obtained. Of the two alkalies potassium is the more preferable when a constant concentration is to be employed, because of the anesthetic action of the potassium ion as well as the potentiation of the alkali. When equal mixtures of alkali and novocaine are employed sodium seems to be the equal of potassium.

An attempt was made to determine the influence of the solvent by comparing mixtures of novocaine and sodium bicarbonate in distilled water with the same mixtures in physiologic salt solution. These results expressed in Table IV indicate slightly better action with distilled water as the solvent. This difference might be within the limits of experimental error, but it is quite constant in all dilutions and all values represent the averages of several trials.

Discussion.—This method has been criticized because it is a determination of motor nerve block and therefore does not measure the efficiency on sensory fibers. It should be of comparative value, however, for the velocity of block of motor nerves is a fair indication of comparative action on sensory nerves, the latter being affected more easily. The results cannot be transferred to clinical practice since the conditions of absorption are not reproduced. On the other hand the accuracy of measurement is not influenced by many factors which are present in the living, such as the presence of the solution within the tissues, its tonicity and diffusibility and the site and mode of injection.

The data of different investigators using this method have varied widely. Absence of response to stimulation depends, as shown in these experiments upon the solvent, the strength of the stimulating current and especially upon the distance of the point stimulated from the end of the nerve. Sollman mentions other factors such as the length of the immersed segment, temperature and size of the nerve trunk. The writer is unable to state whether these factors increase or decrease the velocity of block. Of far greater importance is the individual variation of different frogs. This feature is easily sufficient to account for discrepancies of different investigators. The normal survival time of twenty preparations placed in pans of salt solution and covered with filter paper varied between four and sixteen hours or 400 per cent. Differences in velocity of block of 100 per cent are occasionally seen, and it is not rare to observe the half per cent solution becoming effective before the one per cent. Averages of several trials must therefore be taken, since the factors governing individual variation are unknown. This feature makes the method one of coarse approximation only, and inherently incapable of the refined accuracy usually associated with a scientific quantitative method.

II. BLOCK OF THE SENSORY FIBERS IN THE SCIATIC NERVE OF THE FROG

The efficiency of local anesthetics on sensory fibers of animals is difficult to estimate. The sciatic or brachial nerve of rabbits has been isolated, sprinkled with the anesthetic drug and the time of paralysis observed. The same method has been followed in the determination of the concentration required to produce complete anesthesia, but these methods are not practical for the present study. The method of Sollman of applying the solution to the lumbosacral plexus of the frog is of much greater quantitative value.

Method.—The frogs were decapitated leaving the lower jaw then eviscerated through an incision across the abdomen just below the sternum. The lower abdominal cavity thus forming a little pocket was then filled with small cotton pledgets saturated with anesthetic solution, so that the lumbosacral trunks throughout their course within the abdomen were bathed in the solution. At intervals of from five to ten minutes the persistence of the reflex path was tested by immersing the feet in N/10 hydrochloric acid. According to Sollman disappearance of the reflex indicates destruction of the reflex arc by interruption of conductivity in the sensory fibers. He states that after thirty or forty minutes the paralysis may be due to death of the reflex centers and should not be counted.

At the time of complete disappearance of the reflex the excitability of the motor fibers was tested by stimulation of the plexus. This was successful in every case and remained active for from ten to thirty-five minutes after paralysis of the sensory fibers, the plexus being bathed all the while in

TABLE V

MINUTES REQUIRED FOR COMPLETE BLOCK OF SENSORY FIBERS OF THE FROG'S SCIATIC

	2%	1%	½%	
Physiologic salt solution				80
Tap water				72
Distilled water				52
Quinine urea HCl.	40	84	0	
Novocaine	7	19	29	
Potassium bicarbonate	6	12	37	
Potassium sulphate	5	15	22	
Magnesium sulphate		85		
Sodium chloride		75		
Calcium chloride		70		
Sodium bicarbonate		70		
Gelatine	85			
Sodium sulphate		69		
Chloretone			77	

TABLE VI

ANESTHETIC POWER OF POTENTIATION MIXTURES ON SENSORY FIBERS OF FROG'S SCIATIC

	½% EACH	¼% EACH	⅛% EACH	1/16% EACH
Novocaine and magnesium sulphate	30			
Novocaine and calcium chloride	27			
Novocaine and sodium sulphate	32			
Novocaine and quinine urea hydrochloride	40			
Novocaine and gelatine	35			
Novocaine and potassium sulphate	9	23	45	
Novocaine and potassium bicarbonate	8	15	40	64
Novocaine and sodium bicarbonate	9	14	35	60

anesthetic solution. This action is comparable to the earlier effect upon sensory than motor fibers sometimes seen in a light spinal anesthetic in which a leg is painlessly amputated without motor paralysis, the patient being able to move or raise it himself.

Results.—The survival period of such preparations kept in salt solution was from one and a half to two and a half hours. Contrary to Sollman's findings, when the reflexes had once disappeared they did not return, so that the period of recovery could not be estimated. Preparations were kept in saline for from two to five hours testing for recovery, still no restoration of reflexes was seen. The results expressed in Table V, therefore, indicate velocity of anesthetic action only. Novocaine and potassium salts are equally active. Quinine urea hydrochloride is much less powerful, while chloretone may be classed with the inert salts.

No potentiation was observed by this method except with potassium salts and alkalis. As in paralysis of motor fibers, the potentiation with potassium was less than that of the alkalis (Table VI). Potentiation is stronger when an excess of alkali is used, that of potassium bicarbonate being better than sodium bicarbonate, especially in the higher dilutions (Table VII).

TABLE VII

ANESTHETIC POWER OF NOVOCAINE WITH EXCESS OF ALKALI

NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%
½% Sodium bicarbonate	7	13	30	45	62	77	0
½% Potassium bicarbonate	8	13	16	19	24	24	30

Discussion.—The efficiency of local anesthetics on sensory fibers is difficult to estimate because of the introduction of the complicating factor of the reflex arc. It is aided in this case by removal of the higher nerve centers and destruction of circulation. Paralysis would occur as well with interruption of the reflex pathway at other points than along the afferent fibers, as for example, at the reflex center. This could occur from absorption on the anesthetic drug. Considerable surface is bathed within the frog's abdominal cavity which, with diffusion and lymphatic absorption and the action of lymph hearts might produce a systemic action sufficient to inhibit the reflex centers. This feature is offered as a possible explanation of the failure of reflexes to return.

As a method of quantitative determination of anesthetic action it is unreliable and can only give crude approximations. It is certainly not sufficiently sensitive or constant to be of much clinical value alone and must be considered together with other methods.

III. SURFACE ANESTHESIA OF THE FROG'S SKIN

Application of anesthesia solutions to the skin of the frog is a method more suited to the most powerful anesthetics. Novocaine is of little clinical value as a surface anesthetic, its only use being in the urethra and its value there is questionable. It is not as well suited for anesthesia of mucous membranes as cocaine and a few other substances which penetrate this structure more readily. This method was tried along with the others in the

hope that the potentiation formulas might prove as powerfully anesthetic as cocaine.

Method.—Frogs were decapitated leaving the lower jaw. The viscera were removed and the body cavity washed out. The right leg was then immersed as high as the knee in a test tube of anesthetic solution. At the end of ten-minute intervals both legs were tested by immersion in N/10 HCl about half way to the knees. When both legs were simultaneously withdrawn the substance was ineffective; if the right leg was not withdrawn sensation was absent. The test was then performed on the left leg without control.

Results.—Table VIII shows the comparative activity of the different substances acting alone. Preparations remain active for periods of from one to two hours. The inert salts evidently possess slight anesthetic qualities when sufficiently hypertonic. Potassium salts and novocaine remain about the same, while quinine urea hydrochloride is inferior, as seen in the other tests. The surprising feature is the high efficiency of chloretone in half per cent solution, which is approximately the equal of cocaine in 2 per cent solution. This value for cocaine is much lower than that given by other investigators. The values for individual trials with 2 per cent cocaine varied between five and twenty-five minutes. One value of thirty-five minutes was thrown out. The average value of twelve minutes is near Sollman's value for half per cent novocaine. Recovery periods are not included since recovery is very rarely observed even when washed in tap water.

TABLE VIII

COMPARATIVE ACTIVITY OF SUBSTANCES ACTING ALONE

	4%	2%	
Tap water			105
Physiologic saline			75
Distilled water			85
Gelatine		70	
Sodium chloride	27	31	
Sodium bicarbonate	40	54	
Magnesium sulphate	31	50	
Calcium chloride	50	41	
Sodium sulphate	40	45	
Potassium sulphate	19	45	
Potassium chloride	18	30	
Potassium bicarbonate	20	40	
Quinine urea hydrochloride	35	40	
Chloretone, ½ and ¼%	11	19	
Novocaine	20	45	
Cocaine		12	

Table IX expresses the activity of the potentiation mixtures. The inert salts are of no value. Chloretone and novocaine give simple summation. The same potentiation with alkalis is seen as in the previous methods. Novocaine plus alkali is the equal, if not the peer, of the same strength of cocaine.

Discussion.—Braun criticizes results by this method as applying only to the skin of the frog, the physiologic properties of which must materially influence the local action of the substance. He failed in many instances to observe any stimulation with many very irritating solutions and maintains that a comparison of these results with other methods is not possible as long

TABLE IX

ACTION OF POTENTIATION MIXTURES

NOVOCAINE	ONE PER CENT
1% Magnesium sulphate	30
1% Sodium chloride	30
1% Calcium chloride	28
1% Sodium sulphate	35
1% Potassium chloride	27
1% Potassium sulphate	50
2% Gelatine	45
1% Potassium bicarbonate	17
1% Sodium bicorbonate	13
1% Quinine urea hydrochloride	33

as the permeability of frog's skin for various substances is unknown. We find roughly, with the exception of chloretone, the same ratio of anesthetic action by this method as in I and II and cannot criticize it on that score. Our objection to the method is in the introduction of the complicating factor of the reflex arc and the marked individual variation. Interruption in conductivity of the reflex arc may take place at other points than at the sensory terminals. Sollman states that after forty minutes the results become unreliable because late reactions depend upon the survival period of the reflex centers. This feature would also account for a failure of the reflexes to return. Individual variation of from one to five hundred per cent is often seen. It is this feature which accounts for the marked discrepancies among the different workers. Hamilton[7] states that this method represents a reaction capable of accurate measurement, and as such should take its place as a valuable pharmacologic assay method for establishing relative values. It is true that the reaction can be accurately measured; but when the absence of reaction may depend upon other things than paralysis of sensory terminals and when individual differences due to unknown factors are so marked, results will continue to be quite variable. Gradenwitz[5] observed even finer distinctions with this method than Hamilton. If the leg to be tested was drawn out of the acid before the control leg he assumed that the substance had increased sensibility; if the control leg was drawn out sooner than the leg to be tested it represented a decrease in sensibility. In our experience this method is incapable of such a refined degree of quantitative distinction.

IV. ANESTHESIA OF THE RABBIT'S CORNEA BY IRRIGATION OF THE CONJUNCTIVAL SAC

A common method of testing surface anesthesia is the irrigation of the conjunctival sac. This test involves both penetration of the mucous membrane and action upon nerve terminals. Sollman states that its results can be transferred directly to clinical practice.

Method.—The rabbits were wrapped snugly in a towel or apron leaving the head free. The winking reflexes were tested by touching the cornea with the tip of a lead pencil. The lashes were clipped closely. The lower lid was pinched into a pocket which was filled with solution by means of an eye dropper. The conjunctiva was thus bathed for one minute. At five-minute intervals the winking reflex was tested by touching the cornea with the

point of a lead pencil over a period of twenty minutes. The end point was regarded reached when the pencil could be drawn across the cornea with moderate pressure without causing winking. Rabbits in which the test was positive were not used again on the same day thus allowing ample time for recovery. This is essentially the method described by Sollman.

Results.—The results of individual action of these substances are shown in Table X. The inert salts, potassium salts, chloretone, and quinine urea hydrochloride were all inactive. Novocaine was only questionably positive on two trials and entirely without effect in four. Cocaine anesthetizes absolutely and promptly in 2 per cent solution.

TABLE X

COMPARATIVE ACTION UPON THE CONJUNCTIVA OF THE RABBIT

	4%	2%	½%
Magnesium sulphate	0		
Calcium chloride	0		
Sodium chloride	0		
Sodium sulphate	0		
Sodium bicarbonate	0		
Potassium chloride	0		
Potassium bicarbonate	0		
Potassium sulphate	0		
Quinine urea hydrochloride	0		
Chloretone			0
Novocaine	0		
Cocaine		2+30	

TABLE XI

ACTIVITY OF POTENTIATION MIXTURES

NOVOCAINE	2 PER CENT
2% Magnesium sulphate	0
2% Sodium sulphate	0
2% Calcium chloride	0
2% Potassium sulphate	0
2% Potassium chloride	0
¼% Chloretone	0
2% Quinine-urea hydrochloride	0
2% Potassium bicarbonate	5+25
2% Sodium bicarbonate	5+25

The potentiation mixtures, Table XI, showed no activity in two per cent mixtures with potassium salts. Corneal anesthesia was observed only when novocaine was combined with alkali. This action must be regarded as potentiation since both substances were inactive alone in 4 per cent solution. This constitutes further proof that the addition of the alkali to the novocaine solution increases the power of penetration as well as the anesthetic action.

Discussion.—The attitude of investigators toward the efficiency of this method varies. Sollman is an enthusiastic supporter of the method while Closson finds it unsatisfactory. Our first criticism of its value is the great individual difference in rabbits. Rabbits of a sluggish temperament often tolerate touching of the cornea with the pencil point when no anesthesia is present. We found rabbits which we discarded as unsuitable for the test because of this factor. Rabbits of the opposite temperament, those which resist handling and are alert and excited often wink before the pencil has

touched the cornea even with the cocaine anesthesia. The chloretone solution may have been anesthetic, but it is apparently so irritating as to compromise the determination of an end point. The criteria of the end point are also purely arbitrary and have been applied differently by different investigators. The end point will vary with the amount of pressure employed in drawing the pencil across the cornea, the number of repetitions and whether the point of the pencil is smooth or presents fine gritty material which will scratch the cornea. Winking often occurs sluggishly after repeated stimulation with marked pressure. In such instances one is uncertain whether there is partial anesthesia or absence of anesthesia in an animal of naturally sluggish reactions. As an accurate quantitative test the ophthalmic method is unsatisfactory and suitable only for rough approximations. Even then, results of different investigators will not be comparable and the same ratio of anesthetic activity by the different methods of laboratory investigation may not be constant. For this reason no attempt has been made to compare the efficiency of novocaine plus bicarbonate with cocaine employing a series of dilutions of each.

V. MUCOUS MEMBRANE ANESTHESIA OF THE HUMAN TONGUE BY IMMERSION

Anesthesia of the buccal mucous membranes was one of the earliest local anesthetic effects to be observed. The sensation of numbness resulting from chewing coca leaves suggested its use as a surface anesthetic. Surface anesthesia of unknown solutions may be demonstrated by applying them to the tongue or gums. The anesthetic effect is manifested by a sensation of numbness very easily recognized. It is an attractive method because it is essentially the same as that of clinical practice.

Method.—The tip of the tongue was immersed in the solution for two minutes, after which the mouth was washed out and the tongue tip tested for anesthesia by rubbing it against the cutting margins of the incisor teeth. An attempt was also made to test anesthesia by pricking the tongue tip with a needle but this was unsatisfactory. The needle prick may be painful even with mucous membrane anesthesia. All tests were made upon the writer's tongue. Tests were performed in succession with the inert substances, but when anesthesia resulted it was necessary to wait until there had been absolute recovery. Only a limited number of dilutions were therefore employed

TABLE XII

ACTION UPON THE TONGUE

	2 PER CENT
Sodium bicarbonate	0
Calcium chloride	0
Potassium chloride	0
Potassium sulphate	0
Sodium sulphate	0
Magnesium sulphate	0
Potassium bicarbonate	0
Chloretone ½ per cent	0
Quinine urea hydrochloride	0
Novocaine	0
Cocaine	+11

so that the method would not become irksome and prolonged over an undesirable length of time.

Results.—The comparative activity of substances acting alone is shown in Table XII. None of the substances tested showed any mucous membrane anesthesia whatever, with the exception of cocaine. This promptly produced a numbness lasting eleven minutes, more marked on the under surface of the tongue. As in the tests upon the rabbit's cornea, the superiority of cocaine as a surface anesthetic was easily demonstrated.

TABLE XIII

ACTIVITY OF POTENTIATION MIXTURES

NOVOCAINE	TWO PER CENT
2% Magnesium sulphate	0
2% Sodium sulphate	0
2% Calcium chloride	0
2% Potassium chloride	0
2% Potassium sulphate	0
2% Quinine urea hydrochloride	0
½% Chloretone	0
2% Potassium bicarbonate	+8
2% Sodium bicarbonate	+11

As was to be expected, no anesthetic action was observed in mixtures of novocaine with the inert salts, potassium salts, chloretone and quinine urea hydrochloride. Very marked anesthetic effect was produced by equal mixtures of novocaine and the bicarbonates. It was demonstrated that novocaine plus bicarbonate roughly equals the same strength of cocaine, at least in the 2 per cent dilutions employed.

Discussion.—This method is the most accurate one employed thus far. There is not the question of individual variation. By using the tongue tip the influence of pressure and salivary dilution is avoided. These play a part when pledgets of cotton are placed in the buccomandibular sulcus. Anesthesia does not leave the tongue tip suddenly, but any inaccuracy that might result from this feature is obviated by the fact that all tests were made on the same individual over a period of several days so that he learned to use the same criteria of recovery in all cases.

VI-*a*. ANESTHESIA OF THE DOG'S SKIN BY DERMAL WHEALS

Subcutaneous infiltration in the dog has been employed by Pittinger[20] as a biologic method for the quantitative evaluation of local anesthetics. He injected 1 c.c. of solution at a single puncture and tested sensation in the overlying skin with a red hot wire. He claims to have determined that cocaine was eleven times as active as novocaine by this method. It has long been pointed out that subcutaneous tissues are not suitable for such experiments because they are not sufficiently sensitive, and since they are not situated on the surface of the body it is impossible to determine the disturbance of sensation from the substance injected but only that of the overlying skin. We have modified this procedure by substituting intracutaneous infiltration for subcutaneous injection. It is then possible to apply the test for sensation at the exact point where the solution exerts its action.

Method.—The hair was closely clipped from an area on the dog's back, then removed completely by dissolving with barium sulphide depilatory mixture. Dermal wheals one-half inch in diameter were then raised in this area, using the Meeker regional anesthesia syringe and finest hypodermic needle. One-fourth of a cubic centimeter of solution was employed in each wheal and extreme care taken so that the infiltration was entirely intracutaneous. By means of a red hot wire the wheals were then tested at intervals for the return of sensation.

Results.—To those who have had experience in animal experimentation the difficulty in interpretation of sensations is well known. It is obvious that such results are only of questionable value. The data expressed in Tables

TABLE XIV

ANESTHETIC VALUE OF SUBSTANCES ACTING ALONE

	2%	1%	½%	
Distilled water				11
0.9% salt solution				5
Magnesium sulphate		5		
Sodium sulphate		10		
Calcium chloride		10		
Gelatine	15			
Potassium chloride		8		
Potassium sulphate		15		
Potassium bicarbonate		10		
Sodium bicarbonate		15		
Novocaine		18		
Cocaine		30		
Quinine urea hydrochloride				

TABLE XV

ANESTHETIC VALUE OF POTENTIATION MIXTURES

	1% EACH
Novocaine and gelatine	24
Novocaine and sodium bicarbonate	20
Novocaine and chloretone (¼%)	22
Novocaine and quinine urea hydrochloride	32
Novocaine and magnesium sulphate	15
Novocaine and potassium chloride	22
Novocaine and potassium bicarbonate	24
Novocaine and potassium sulphate	19
Novocaine and sodium sulphate	15

XIV and XV represent experiments performed upon twelve dogs. Only gentle dogs were selected. They were not tied down or restrained in any manner, but were usually blindfolded, held upon the lap of an assistant and kept quiet by petting. No restraint was necessary even during the intracutaneous injection. Evidence of pain was manifested in a variety of ways, such as straining, whining, wiggling or shaking the skin by action of the platysma muscle. The most reliable manifestation was that of shaking the skin or wincing, while the animal remained otherwise quiet. These arbitrary manifestations were extremely variable, difficult to interpret and inaccurate. For example, dog No. 3 was very tame and lay quietly in an assistant's lap. All wheals were raised without any resistance. When stimulation over a wheal of salt solution produced no pain he was stimulated in normal skin. He tolerated the burn just as well in unanesthetized skin as in a

wheal of novocaine, until almost the entire thickness of the skin was burned through. He was then less valuable for subsequent tests because one could not tell whether he complained at stimulation of a wheal or at pain from the first burn in unanesthetized skin.

Dog 11 was of a different nature. Wheals were raised during considerable struggling. Later he winced and struggled at all forms of stimulation, even touch with a lead pencil. When a fresh wheal of cocaine was stimulated with the red hot wire he cried out loudly, although he could not possibly have felt any other sensation than moderate pressure. In another dog wheals regarded sensitive ten minutes after infiltration were repeated and found to be entirely insensitive twenty minutes after infiltration. These are a few of the difficulties encountered in trying to interpret a dog's sensations.

Discussion.—While experimentation on dogs is of value in the determination of local irritation and toxicity of anesthetic drugs, it furnishes very questionable data on painful sensations. A dog may either suffer patiently without external manifestation or struggle, strain, whine or cry out in dissatisfaction at its treatment similar to manifestations of actual pain. Such actions are more likely to occur when the animal is being fussed over. Rather than consider this method one of accurate pharmacologic assay we maintain that it has exceedingly little value. While Pittinger determined cocaine to be eleven times as powerful in anesthetic value as novocaine we were usually unable to distinguish novocaine from cocaine. His results are entirely at variance with those of other investigators using the human skin, so that we can only conclude that the method should be discarded as erroneous for quantitative determinations.

VI-*b*. ANESTHESIA OF HUMAN SKIN BY DERMAL WHEALS

Anesthesia of human skin by dermal wheals is an attractive method because it is identical to clinical usage. The patient is allowed to judge in all cases and interpret his own sensations. The great advantage of this method over others was first emphasized by Braun, who determined anesthetic strength both by duration of anesthesia and by minimal effective concentration. It has been shown by several surgeons to give the nearest approach to absolute anesthetic power.

Method.—The thighs and anterior abdominal wall were closely shaved. Dermal wheals were then raised with the Meeker syringe and finest hypodermic needles freshly sharpened. The needle was thrust beneath the epidermis parallel to the skin surface with the bevel downward. At the moment the point of the needle was buried in the epidermis injection began, which was always endermic and not subcutaneous. Dermal wheals were thus made to stand up from the surrounding skin surface like an urticarial wheal. Wheals were one-half of an inch in diameter and required one-fourth of a cubic centimeter of solution each. It is important that all wheals be as nearly as possible the same size and contain the same amount of solution, all of which has been injected intracutaneously. It is also necessary that adequate control wheals of salt solution and of novocaine be tested together with the

unknown mixture, otherwise a distorted sensation might be interpreted as anesthesia.

All wheals were made upon the author by himself, most of them on the anterior surfaces of the thighs from knees to hips. The skin in this region is of sufficient thickness that wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin as well as the rapidity of absorption varies in different areas of the body. It also varies in different individuals, depending upon familial traits, exposure and muscular exercise. By employing the same skin areas in the same individual these factors remained constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating or muscular exercise because of the improved circulation and consequent rapid absorption. In these tests the patient remained seated and sources of external heat were avoided. Wheals were marked with a circle of mercurochrome as soon as raised so that the center of the endermic infiltration was easily identified after the wheal had disappeared.

TABLE XVI

ANESTHETIC VALUE OF SUBSTANCES ACTING SEPARATELY

	AQ. DIST.		PHYSIOL. SALT SOLUTION								
	1%	½%	1%	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%	
Physiologic salt solution											0
0.45% salt solution											0
Distilled water											0
Magnesium sulphate	0		0	0							
Sodium sulphate	0		0	0	0						
Sodium bicarbonate	0		0	0							
Calcium chloride	18		25	3†	0						
Potassium bicarbonate	18		27	3†	0						
Potassium sulphate	20		28	5†	0						
Potassium chloride	19		25	5†	0						
Gelatine	15		0	0							
Chloretone		28		18	5†	0					
Quinine urea hydrochloride	160		95	52	40	28	11†	0	0		
Novocaine	33		28	20	15	8	5	0			
Cocaine	45		32	27	20	16	12	8	5†	0	

Other investigators have tested for anesthesia by means of a fine cotton wisp. The stroking of the area with a wooden applicator or toothpick, or scratching with a needle as in vaccination was found to be more accurate. By means of the cotton wisp disturbed or distorted sensation as in a wheal of physiologic salt solution may be mistaken for anesthesia. Of much greater importance than the manner of testing is the employment of adequate control wheals with the series of dilutions of the unknown solution.

Results.—Table XVI expresses the action of each substance alone. Magnesium sulphate, sodium sulphate and sodium bicarbonate possess no anesthetic value but are similar in effect to wheals of physiologic salt solution. Calcium chloride was found to be anesthetic in 1 per cent solution, although all previous tests had shown it to be inert. The three potassium salts are about equal in anesthetic action. In previous tests they were almost the equal of novocaine, but in dermal wheals they are much inferior. Gelatine was anesthetic in distilled water but not in salt solution. Chloretone was

inferior to novocaine and cocaine, while quinine urea hydrochloride was the superior in higher concentrations.

There was no potentiation with any of the mixtures tried (Table XVII). The duration of anesthesia with the mixtures was approximately the equal of the same strength of novocaine acting alone. There was not even summation of action with potassium salts in ½ per cent strength, and the addition of bicarbonate did not appreciably improve the solution. Chloretone and novocaine give summation only. The addition of quinine urea hydrochloride prolongs anesthesia by imperfect summation and not potentiation.

While the specific action of a substance should be studied when the solution is isotonic, valuable information is also obtained when distilled water is used as the solvent. Studied in this manner many substances are decidedly painful before their anesthetic action is established, although the true local anesthetics as novocaine and cocaine in strengths sufficient to anesthetize are entirely painless to infiltration. An anesthetic producing pain for its injection has been called "anesthetica dolorosa." Distilled water is very painful and only partially anesthetic for a short period. Some surgeons claim to have performed operations painlessly by means of copious injections of water but certainly the very painful infiltration and tumefaction with imperfect anesthesia must have afforded but little more palliation than operation without any anesthesia whatever. The fact that injections of water have been observed to bring about good results in such painful affections as sciatica and neuralgia may be explained upon another basis than the supposed nerve blocking properties of the water.

An attempt to group these substances according to the amount of pain produced shows the following relationship:

TABLE XVII

ANESTHETIC VALUE OF POTENTIATION MIXTURES

	½%	¼%	⅛%	1/16%
Novocaine and sodium sulphate	19	14	9?	
Novocaine and magnesium sulphate	20	17	6?	
Novocaine and calcium chloride	20	18	11?	
Novocaine and potassium chloride	21	17	10?	
Novocaine and potassium sulphate	18	13	9?	
Novocaine and potassium bicarbonate	18	7	5	
Novocaine and sodium bicarbonate	21	15	11?	
Novocaine and chloretone		17	12	6?
Novocaine and quinine urea hydrochloride	64	40	27	18

1% Cocaine	0.
1% Novocaine	0.
1% Quinine urea hydrochloride	+++.
¼% Chloretone	++.
1% Gelatine	++++.
1% Potassium chloride	++++.
1% Potassium sulphate	++++.
1% Potassium bicarbonate	++++.
1% Calcium chloride	+.
1% Sodium sulphate	++.
1% Sodium bicarbonate	++.
1% Magnesium sulphate	+.
.9% Sodium chloride	+.
.45% Sodium chloride	++.
Distilled water	++++.

Potassium salts and quinine urea hydrochloride were very painful before anesthetic, thus showing the necessity for the same tonicity of the fluid injected as that of the tissues.

To determine the influence of gelatine, dilutions of novocaine were used in a 4 per cent gelatine medium. Injections were made in three series. In one the gelatine was sterilized by boiling for five minutes. In the two remaining series the mixtures were not boiled. One of these was physiologic salt solution and the other distilled water. The three series were approximately equally anesthetic and only questionably superior to the action of novocaine alone. Absorption was not appreciably delayed by the use of gelatine. (Table XVIII.) This confirms and extends Braun's observations with sterilized gelatine solutions. Even though gelatine improved the novocaine solution, the fact that it is an excellent bacteriologic culture medium and congeals in solutions above 2 per cent at room temperature, would prohibit its use as adjuvant to local anesthesia.

TABLE XVIII

ACTION OF NOVOCAINE IN GELATINE SOLUTION

NOVOCAINE	½%	¼%	⅛%	1/16%
4% gelatine in salt sol. sterilized by boiling	24	19	11	5?
4% gelatine in salt sol. unsterilized	25	20	17	13?
4% gelatine in aq. dist. unsterilized	25	21	13	6?

TABLE XIX

INFLUENCE OF ADRENALIN UPON ANESTHETIC ACTIVITY

	1%	½%	¼%	⅛%	1/16%	1/32%
Novocaine	28	20	15	8	5	0
Novocaine and adrenalin (10 drops per 100 c.c.)		120	82	43	10	?

The addition of ten drops of adrenalin (Parke Davis, 1 : 1000) to the hundred c.c. of anesthetic solution prolongs anesthesia in a very decisive manner. There is not true potentiation because the minimal effective concentration is not altered. (Table XIX.) The effect has been repeatedly shown to be due to the vasoconstriction of the adrenalin which has earned it the name of the "chemical tourniquet." The value of this agent is universally recognized, so that the only excuse for including it in this series of observations is for comparison with the other drugs employed for synergistic action.

Discussion.—This method involves direct action on the terminal nerve filaments and sensory end organs. The complicating factor of penetration is not present, since the force in injection produces diffusion of the solution. In well-formed wheals the solution is confined to the upper layers of the skin and is never below the derma proper. Anesthesia does not extend beyond the limits of the raised margin, and sensation returns from the periphery toward the center. When wheals are accurately placed, a sufficient number of controls employed and when the patient is experienced in the interpretation of the end point, this method is easily the most valuable one for quantitative estimation of local anesthetic power.

GENERAL DISCUSSION

A comparison of results obtained by the different methods shows that there is considerable variation. Determinations of local anesthetic action by the methods employing decapitated frogs must always be discarded when they do not agree with data on the human.[19] In addition to the great difference of station in the scale of animal life is the fact that experimental conditions do not correspond to those of clinical usage. The action of the frog's sciatic nerve has been compared to nerve blocking in surgery. But the frog is first killed, the nerve trunk isolated, immersed in the anesthetic solution and the end point determined by motor response. There is not the problem of diffusion of the solution through tissues surrounding the nerve trunk and the influence of anemia, pressure and edema, and the absorption of the anesthetic medium, all of which are of great importance in clinical usage.

The second method, the action upon the sensory fibers of the lumbosacral plexus is open to many of the same objections. The fact that the frogs were first killed and that the reflex arc had to be employed in determining the presence of sensation render it worthless when results do not agree with those obtained upon man.

The third method, anesthesia of the frog's skin, is not to be compared to mucous membrane anesthesia in man. It has been maintained that because the frog's skin is wet, conditions of mucous membrane anesthesia in man are closely reproduced. As a matter of fact there is a vast difference both anatomically and physiologically which would indicate a different permeability ratio. By this method chloretone was far the superior of cocaine, while it is of no value at all in surface anesthesia in man. As a sole method of evaluating local anesthetics, killed frogs would give erroneous data. Their continued use for this purpose represents merely a heritage from older physiology.

All three experiments on frogs showed potentiation with potassium and alkalies, more marked with the latter. Potentiation with alkali was also quite marked in surface anesthesia both on the cornea of the rabbit and on the human tongue. These methods are identical to clinical use and suggest the addition of alkali to novocaine solutions for mucous membrane anesthesia. The author plans to give the method a clinical trial particularly in urethral anesthesia.

It seems certain that the improvement of alkali consists in increased ability to penetrate mucous membranes. This same power of penetration would be of immense value in nerve block and field block. In field block fanwise injections are made in such a manner in the same plane that intervening tissues are penetrated by the solution to form a continuous wall interrupting conductivity of all nerve filaments passing through it. While there is no direct experimental data bearing on this procedure, results with dermal wheals as well as clinical experience indicate no benefit from the addition of alkali. Granting that free bases penetrate more readily than the salts Sollman states that in surgical use the anesthetics do not reach the nerve filaments as pure salts, but that they are partly converted into carbon-

ates by the alkalinity of the tissues. The importance of alkalization would thus be insignificant in nerve block and field block.

An attempt was made to determine experimentally the effect of alkali upon penetration. Small blocks of various tissues, both fresh and formalin hardened, were placed in two solutions. The solutions consisted of 2 per cent novocaine in physiologic salt solution colored with methylene-blue, one of which also contained 2 per cent sodium bicarbonate. After two hours blocks of tissue were cut across and the thickness of colored margin measured. No difference in penetration was observed at the end of this time. The method is open to objection in that conditions of absorption, pressure and edema in the living are absent. The thickness of the colored margin may also indicate penetration of the dye only and not that of the anesthetic drug.

A surprising feature of the results by dermal wheals in man was the comparative inefficiency of potassium salts. In the earlier methods these were the equivalent of novocaine, and mixtures of the two showed definite synergistic action. In man potassium salts cannot be distinguished from calcium chloride and there is no value at all either in potentiation or prolongation of anesthetic action by their addition to the novocaine solution in strengths suitable for clinical use.

It has been repeatedly shown that there is no advantage in employing mixtures of the local anesthetic drugs, since only summation and not potentiation results. Anesthetic action may be prolonged by the addition of quinine urea hydrochloride to the novocaine solution, but not without the undesirable local tissue effect of the quinine salt. Certainly the prolonged anesthetic action of quinine urea hydrochloride in sufficient strength is due to a destructive effect upon tissues, in some instances resembling coagulation necrosis. The writer can exhibit marks of several wheals on his body as evidence of this fact. Any benefit in prolonged anesthetic action from the addition of quinine urea hydrochloride is thus overcome by the undesirable local tissue effects.

The same objections apply to mixtures of novocaine and chloretone, although the latter is by no means as irritating locally as quinine. While local anesthetic action is prolonged by the use of a saturated solution of chloretone as the novocaine solvent, it is not a novocaine effect alone but a summation of actions of the two drugs. Since chloretone alone has never found favor in local anesthetic work, its addition to the novocaine solution to accomplish something more efficiently done by doubling the amount of novocaine is not to be recommended. The only remaining method of improving the action of novocaine in isotonic salt solution is by the addition of adrenalin. This improvement is so definite and decisive that the questionable value of the other proposed ingredients dwindles into insignificance. These experimental results are in agreement with the vast majority of clinical experience with local anesthetic methods.

The current methods of estimating anesthetic efficiency are simple in principle and execution, but those performed upon laboratory animals are of fundamental importance only when they agree with results obtained upon man. If the results of testing the different local anesthetics by the different

methods agreed relatively, then comparison by any one of the methods would give relative efficiencies. The varying results of these experiments as well as the varying ratios of different investigators show this to be untrue. It has been suggested that animal experimentation may render considerable practical service by cutting short the career of distinctly unpromising local anesthetics before they are introduced into clinical practice. This should apply more to toxicity and local irritating effects. When nontoxic and non-irritating in experimental work, the anesthetic power may be tried at once in experiments upon man. The ultimate efficiency of any new drug or mixture will then depend upon its action in practical use.

CONCLUSIONS

1. The current methods of estimating anesthetic efficiency upon laboratory animals are of value only when they agree with experimental results obtained upon man. Animal experimentation is more appropriate for the determination of toxicity and local tissue reaction. When these features are satisfactory the anesthetic power of any proposed local anesthetic drug may be determined at once by experimentation upon man.

2. Experimentation with decapitated frogs ranks lowest in value among these laboratory methods. In addition to the great difference of station in the scale of animal life is the fact that experimental conditions do not correspond to those of surgical practice.

3. Of the various substances proposed for the improvement of the novocaine solution, magnesium sulphate, sodium sulphate, sodium bicarbonate and gelatine have no local anesthetic action alone. Calcium chloride, and potassium salts are weakly anesthetic. Chloretone and quinine urea hydrochloride are recognized as local anesthetic drugs.

4. In surface anesthesia true potentiation was observed only by the addition of alkali, either sodium or potassium bicarbonate, to the novocaine solution. By this means novocaine can be made almost the equal of cocaine in mucous membrane anesthesia. While this feature has been pointed out by other investigators, the novocaine-bicarbonate mixture seems not to have been employed in clinical use. It is recommended that the mixture be given a trial in mucous membrane anesthesia of the eye, nose and throat, and urethra.

5. For injection anesthesia none of the substances proposed for improvement seem to have sufficient value to warrant their use. This is in marked contrast to the decisive prolongation of anesthetic action observed by the addition of adrenalin to the novocaine solution. Experimental results in this respect agree with the vast majority of clinical experience with local anesthetic methods.

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EFFECT OF CHLORINE TREATMENT AS GIVEN AT EDGEWOOD ARSENAL, MARYLAND, ON THE LEUCOCYTE COUNT*

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VEDDER and Sawyer,[1] in explaining the beneficial effects of chlorine on common colds, whooping cough, and certain other respiratory infections of the upper respiratory tract, attributed at least part of this curative action to an increase of polymorphonuclear leucocytes in the affected mucous membranes and to a stimulation of phagocytosis.

An attempt was therefore made to determine whether the administration of chlorine caused a change in the leucocytes. Since the taking of blood from the mucous membranes of the respiratory tract is scarcely practicable, the work was confined to leucocyte counts of the peripheral blood.

The chlorine treatment was given twice a day, at 10:00 A.M. and at 1:00 P.M. in a large wooden chamber accommodating twenty people. The mixture of air and chlorine was drawn through the chamber at a rate to give a concentration of between 0.014 and 0.015 mg. per liter of air. The ordinary treatment was for one hour. A leucocyte count and a smear for a differential count were made on patients immediately before they entered the chlorine chamber, immediately after the hour's treatment and as nearly as possible one hour later. Counts were made on all persons available for this third count. They included sufferers from acute coryza, bronchitis, sinusitis and a few normal persons. The data were collected over a period of six months, from September to February, the largest number of cases being secured in the winter months. One hundred cases were studied.

The leucocyte count in 25 cases of whooping cough was also studied, but as these patients came from some distance to take the treatment, they were not available for a third count and only the counts immediately before and immediately after the treatment could be secured.

SUMMARY

1. From the tables it may be seen that in 68 per cent of the cases of cold or mild influenza the second count, that immediately following the exposure, was lower than the initial count; in 27 per cent of the cases it was higher; in 4 per cent it was the same as the first count. In 89 per cent of the cases the leucocyte counts were made on patients taking the treatment in the afternoon. The counts made in the morning showed a drop in only 50 per cent of the cases. This suggests that the larger number of decreased counts in the afternoon cases might be due to a natural subsidence from an initial digestive leucocytosis. Forty-eight per cent of all counts, however, showed a drop of over 1000, which should eliminate physiologic factors. It was in

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TABLE I

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	One Hour Later
A	8-23	9	4,800	4,200	4,300			
D	8-26	1:10	8,300	8,200	9,400			
A	"	"	7,600	6,400	8,000			
E	8-29	"	12,000	10,600	18,400			
A	"	"	5,200	6,000	6,800			
L	9-9	"	8,000	6,800	6,600			
L	9-10	"	8,600	8,000	8,000			
S	"	"	9,600	8,800	9,800			
V	"	"	10,400	6,800	8,300			
V	9-15	"	9,800	7,000	7,000			
S	9-17	"	9,700	8,200	8,800			
M	9-26	"	6,600	4,200	5,000			
A	10-10	"	6,800	7,400	6,600			
S	10-22	"	9,200	9,600	8,600			
W	10-23	"	7,800	8,000	8,000			
S	10-24	"	8,600	8,600	8,200	(54-35-6-4-1)	(69-21-4-5-0)	(60-30-4-6-0)
M	10-24	"	7,200	8,500	8,700			
M	11-6	"	10,600	10,600	10,000			
E	"	"	6,500	6,100	7,000			
D	11-13	"	7,200	6,600	6,800			
N	"	"	9,300	9,700	12,600			
N	11-14	"	10,000	9,600	11,000			
K	"	"	8,600	8,000	6,800			
C	"	"	8,600	10,200	9,600			
D	11-17	"	11,200	12,000	8,800			
D	11-18	"	10,200	8,800	7,400			
S	11-19	"	8,000	8,400	6,600			
W	11-21	"	7,800	7,600	9,200	(52-33-7-6-1)	(56-31-9-3-0)	(58-32-3-5-1)
K	"	"	4,600	4,600	5,000			
S	11-22	"	8,600	6,000	8,260			
A	"	"	9,600	10,600	9,600			
O	"	"	5,400	5,000	6,600			
R	11-24	"	7,000	6,000	7,600			

TABLE I—CONT'D

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	ONE HOUR LATER
A	11:24	1:10	6,900	8,000	8,000			
H	"	"	8,200	10,000	8,800			
D	11-26	10:15	8,600	8,200	8,600	(63-28-7-1-0)	(63-31-4-1-1)	(67-30-2-0-1)
S	"	"	8,800	10,200	10,000			
A	"	"	7,400	8,200	10,000	(53-24-20-1-2)	(62-28-10-0-0)	(65-26-8-1-0)
L	11-28	10:00	6,200	5,500	7,400	(63-30-6-1-0)	(64-24-11-1-.3)	(67-22-9-1-1)
P	"	1:30	8,900	8,000	8,200			
C	12-1	"	7,600	7,000	9,400			
W	"	"	10,400	8,400	13,000			
W	12-2	10:00	14,000	12,200	10,000			
C	"	1:30	9,200	6,200	9,600			
P	"	2:00	8,800	5,000	7,800	(70-16-11-2-0)	(72-12-14-1-1)	(55-32-12-1-0)
W	12-3	1:30	8,400	8,200	6,800	(67-25-6-5-1)	(63-15-20-0-2)	
P	12-4	1:30	7,000	8,400	8,000			
M	"	"	6,600	7,600	6,800			
A	"	10:00	6,600	8,400	10,000			
A	12-5	11:00	8,200	9,600	9,800			
R	12-8	1:15	17,000	9,000	9,000	(67-28-4-.5-1)	(66-30-2-2-.4)	(58-40-1-.5-.5)
M	"	"	14,200	8,200	7,800	(57-38-4-0-1)	(64-32-3-.5-.5)	(63-32-3-0-2)
H	12-10	1:30	22,000	8,300	16,800	(56-28.5-14-.5-1)	(61-30-8-1-0)	(61-31-7.5-0-.5)
E	12-12	"	22,400	7,000	7,400	(68-16-15-0-1)	(67-21-9-1-1)	(62-22-9-5-1)
C	"	"	18,800	9,800	9,200	(78.4-18-3.6-0-0)	(60-30-10)	(67-21-11-0-.3)
S	"	"	7,400	7,200	6,400	(67-26-6.7-0-.3)	(70-17-10-2-0)	(68-11-19-1-1)
C	12-13	"	8,000	8,200	9,400	(80-16-4-0-0)	(69-19-12-0-1)	(72-20-7.5-0-.5)
B	"	"	10,400	7,400	7,800	(66-27-6-0-1)	(77-9-12-0-1)	(70-24-4-0-2)
B	12-15	"	11,200	9,600	11,900	(78-15-7-0-.5)	(76-17-5-.5-.5)	
B	12-16	"	11,000	9,400	7,000	(73-22-3-.5-.5)	(75-18.5-4.5-1-.5)	(75-22-1-1-.5)
H	12-16	"	10,800	7,600	8,600	(68-26-5-0-1)	(66-29-2-3-0)	(71-25-4-0-0)
M	"	"	8,000	11,000	7,000	(59-29-10-1-1)	(57-31-10-1-1)	(61-30-6-2-1)
N	12-17	"	16,000	8,000	9,600	(69-20-8-2-1)	(69-25-5-1-0)	(62-29.5-6-2-.6)
L	12-18	"	16,000	11,000	14,000	(66-27-5-1-1)	(64-25.5-9-1-.5)	(65-27-7-.5-.5)
E	"	"	23,200	8,600	16,000	(58-27-13-1-0)	(69-18-12-.5-.5)	(55-26-17-1-0)
N	"	"	13,400	7,000	12,800	(62-24-11-1-1)		(55-32.5-11-1-.5)
W	12-19	1:20	12,600	12,600	10,600	(67-27-6-0-0)	(72-23-4-.3-0)	(61-34-5-.3-0)

TABLE I—CONT'D

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	One Hour Later
A	12-27	1:20	15,600	14,800	14,400	(60-32-7-0-.3)	(61-30-7.5-.5-0)	(66-26-7-.5-0)
W	12-29	1:40	8,000	8,000	6,400	(71-21-7-.3-1)	(63-27-7-2-.3)	(61-29-9-1-0)
A	12-31	1:30	8,200	8,600	9,000	(62-32-5-0-0)	(53-40-6-0-.2)	(54-39-7-0-.3)
A	12-30	1:45	20,200	19,400	13,200	(63-27-9-0-.3)	(75-14-10-.5-0)	(70-25-4-0-0)
A	1-5	1:30	8,600	6,800	6,900	(66-26-7-0-1)	(69-25-5-0-.2)	(62-31-6-0-.3)
M	1-5	2:00	15,400	15,000	13,400	(72-18-7-3-.3)	(80-14-6-.3-0)	(77-15-5-3-0)
A	1-6	1:30	7,800	6,600	7,800	(69-25-5-1-0)	(70-20-9-1-0)	(67-24-7-1-1)
M	"	2:00	12,200	14,200	16,600	(66-22-8-4-0)	(73-17-7-3-0)	(67-20-9-3-.3)
C	1-8	"	6,400	8,200	9,800	(52-42-4-2-0)	(54-40-5-1-0)	(53-40-6-.4-.6)
D	"	"	6,100	10,000	7,600	(57-32-3-5-2)	(50-40-9-0-.5)	(57-28-11-3-1)
A	1-9	1:20	9,400	8,400	10,000	(72-20-7-0-1)	(73-20-6-.5-.2)	(76-19-4-.5-0)
F	"	"	14,200	11,400	7,200	(60-30-8-2-0)	(62-31-5-1-.3)	(60-32-6-2-0)
D	"	"	12,600	7,000	8,400	(64-31-5-.3-0)	(58-34-4-4-0)	(60-31-8-.3-.7)
C	"	"	12,200	10,800	8,800	(64-24-10-1-0)	(60-30-8-1-0)	(60-28-10-1.6-.4)
A	1-10	1:45	6,800	6,200	7,200	(70-23-6-.3-0)	(64-26-8-1.5-.3)	(64-27-8-.7-.3)
M	"	"	13,000	11,800	12,000	(71-24-1-4-0)	(75-15-5-3-1)	(72-18)
C	"	"	11,800	13,200	14,200	(79-14-4-0-2)	(73-18-8-.4-0)	(76-14-8.5-1)
W	1-13	"	14,600	13,800	10,600	(64-28-6-1-1)	(63-28-8-.3-0)	(64-26-9-.8-.2)
M	1-13	"	8,600	7,400	7,000	(57-37-5-1-0)	(56-36-7-1-0)	(56-36-7-.5-.5)
M	1-17	"	17,200	14,400	16,000	(74-19-4-3-0)	(66-21-10-3-0)	(72-23-3-2-0)
W	1-23	2:00	11,800	10,400	12,400	(69-18-9-3-.3)	(74-21-5-0-0)	(76-17-5-1-0)
W	1-24	"	14,000	10,400	11,400	(60-27-9-2.5-.4)	(61-32-2-4-.6)	(57-35-6-2-.3)
S	1-31	1:30	13,200	9,000	8,200			
S	2-2	"	10,800	6,800	7,000	(57-38-3-1-.5)	(51-40-7-1-0)	(51-37-10-1.5-0)
S	2-4	"	7,600	5,200	5,000			
A	2-5	1:15	6,000	6,000	5,300			
D	2-5	1:15	17,000	14,000	14,000	(72-23-4-0-1)	(77-22-1-0-0)	(66-28-6-0-0)
W	2-6	9:45	6,400	7,200	8,200	(50-38-9-1-1)	(54-39-5-2-0)	(47-43-8-1-.2)
D	2-7	1:50	19,000	15,000	11,000	(85-11-3-.6-0)	(85-13-1.8-0-0)	(79-16-4-1-.1)
M	2-9	10:00	10,400	10,000	8,200			
D	2-11	10:45	10,800	13,000	11,400	(65-26-6-2-0)	(57-38-4-1-0)	(60-30-8-2-0)
A	2-18	1:30	7,600	7,200	8,600	(72-25-2-.3-0)	(71-23-5-.3-.3)	
M	2-26	"	9,000	6,200	7,000			

TABLE II

WHOOPING COUGH CASES

DATE	PATIENT	HOUR	BEFORE		AFTER	
			Leucocyte Count	Differential Count	Leucocyte Count	Differential Count
Nov. 28	S. M.	2:10	11,600	(60-40)	10,600	(75-25)
Dec. 1	" "	"	13,400	(78-22)	12,000	(62-38)
Nov. 29	N. M.	"	14,900	(70-30)	11,800	(70-30)
Dec. 1	" "	"	15,000	(55-45)	13,800	(77-23)
Nov. 29	M. M.	"	20,600	(33-66)	12,000	(30-70)
Dec. 1	" "	"	23,800	(55-45)	15,800	(42-58)
Dec. 8	P. M.	1:30	27,800	(17-72-9-1-1)	20,400	(12-83-2-1-1)
Dec. 8	J. M.	"	18,400	(35-63-2-0-0)	18,600	(16-80-1.5-1-1.5)
Dec. 8	P. M.	"	40,400	(25-65-5-5-0)	36,400	(40-56-3-.5-0)
Dec. 8	F. M.	"	21,600	(54-38-6-1-1)	16,000	(28-70-2-0-0)
Dec. 9	" "	"	14,800	(50-44-6-0-0)	11,400	(25-62-12.5-.5-0)
Dec. 9	P. M.	"	26,200	(39-60-0-1-0)	31,800	(38-54-4.5-3.5-0)
Dec. 9	" "	"	16,400	(15-59-25-1-0)	21,800	(16-73-9-1-1)
Dec. 9	R. B.	"	22,000	(31-61-4-3.5-.5)	16,000	(27-65-4-3.5-5)
Dec. 10	" "	"	17,800	(45-49-6-0-0)	16,800	(42-51-6-0-.5)
Dec. 15	M. H.	2:00	33,800	(60-35-3-0-1)	32,000	(58-34-7-0-0)
Dec. 17	R. B.	"	16,000	(40-52-8-0-.5)	13,400	(43-42-12-1-.2)
Dec. 19	M. H.	"	28,000	(34-62-3-1-0)	19,000	(32-57-10-0-.5)
Dec. 19	B. L.	"	22,000	(33-52-13-1-.5)	17,000	(42-49-7-.7-.3)
Dec. 20	R. B.	2:10	19,500	(37-51-12-0-0)	16,000	(54-37-9-0-.3)
Dec. 20	B. L.	1:45	15,000	(44-49-6-1-.5)	12,300	(40-52-7-.3-0)
Jan. 21	E. S.	2:00	24,500	(40-54-5-.6-0)	21,500	(39-52-7-.4-2)
Feb. 19	W. C.	11:20	16,000	(34-57-5-3-1)	14,400	(31-61-4-3-1)
Feb. 19	N. S.	"	26,000	(34-60-5-1-0)	26,000	(32-58-5-4-0)
Feb. 19	R. S.	"	14,200	(64-28-4-4-.5)	13,200	(48-37-6-4-3)

the cases showing a leucocytosis originally that the fall was most marked and in 85 per cent of the cases having an original count of over 10,000 there was a decrease, averaging 4,000. The differential count was only slightly changed, 27 cases having a rise in polymorphonuclears, 23 cases showing a drop and 3 showing no change in polymorphonuclears.

2. Of the 25 cases of whooping cough, 20 or 80 per cent showed a decrease in total leucocytes after the hour of treatment. The average count in these cases was 20,465 before treatment and 15,920 after treatment, or an average decrease of 4,545. Of these cases 17 showed a drop in polymorphonuclear leucocytes averaging 8.9 per cent.

DISCUSSION

The interpretation of these changes is by no means certain, yet it seems probable that the decrease in the peripheral blood may be caused by the migration of leucocytes to the point of irritation, namely, the mucous membranes of the respiratory tract. The migration of from 2000 to 4000 leucocytes in each c.c. of blood to this limited area would lead to a concentration of leucocytes in these parts which should be beneficial to the patient. However, there was no directly apparent connection between the extent of the drop and the degree of improvement. This is undoubtedly to be explained by the fact that the action of chlorine is very complex, and that the increase of leucocytes to the affected part, if it occurs, is only one of five or six factors concerned in the curative action.

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LABORATORY METHODS

A CAGE DEVICE FOR THE STUDY OF KETOSIS AND NITROGEN METABOLISM IN SMALL ANIMALS*

By Harold Levine, B.S., and Arthur H. Smith, Ph.D., New Haven, Conn.

IN metabolism studies on rats recorded in the literature,[1-9] various devices and types of cage have been used to collect the urine. In most instances, the animal rests on a wire screen in a cage, the mesh of which is large enough to permit the urine to drop through and trickle down the sides of a large glass funnel, into a receiving flask placed just beneath the stem of the funnel. By washing down the funnel several times daily, the accuracy of this method of collection is increased. In the apparatus used by Mitchell,[10] the urine drops through a wire screen and is absorbed by filter paper placed in a large crystallizing dish which serves as the bottom of the cage. The above mentioned methods, obviously, are not suited for a study involving a volatile urinary constituent such as acetone. In an effort to surmount this difficulty, a new type of cage, described in this paper, was devised.

Acetone will not volatilize when held beneath a layer of mineral oil. An acetone solution of known concentration was allowed to remain in the cage for a period of six days under a layer of mineral oil five inches in depth. At the end of this period, the acetone solution was analyzed and the result showed that no loss occurred either through volatilization or absorption by the oil. In the cage described below, the principle of urinary collection depends on the capacity of the oil to prevent loss of acetone and on the greater density of the urine as contrasted with the oil.

DESCRIPTION OF CAGE AND METHOD OF COLLECTION OF URINE

Cage Proper.—A diagram of the cage proper is shown in Fig. 1, Part I, with both the feeding (*A*) and drinking (*E*) devices attached. It is cylindrical in shape, 8½″ high and 8¾″ in diameter and constructed of galvanized iron. Ventilation is provided by means of a ¼″ mesh wire screen (*G*) soldered on ½″ from the top of the cage. Small holes (not shown in the diagram, see Fig. 2) ¼″ in diameter, placed at intervals around the cage, 4½″ from the top of the cage, also serve to give proper ventilation.

The feeding device (*A*) consists of a beaker, inside of which a glass food cup (*B*) rests on a rubber stopper (*C*). This beaker is supported by means of a metal ring (*D*), 2½″ in diameter, attached to the side 5¾″ from the top of the cage. In order to have a minimum of scattering by the rat, the food

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receptacle proper (*B*) is placed about 1″ from the top of the beaker. Experiments have shown that rats, as a rule, do not defecate or urinate in this feeding device.

The animal obtains water by means of an inverted bottle (*E*) which rests on the wire screen (*G*) at the top of the cage. From this bottle, a glass tube extends downward into the cage. By licking the end of the tube, the animal receives its water supply.

Urine Collecting Device.—The lower part of the cage which serves to effect both the collection of urine and its separation from feces is shown in Fig. 1, Part II. This funnel-shaped section of the apparatus is also con-

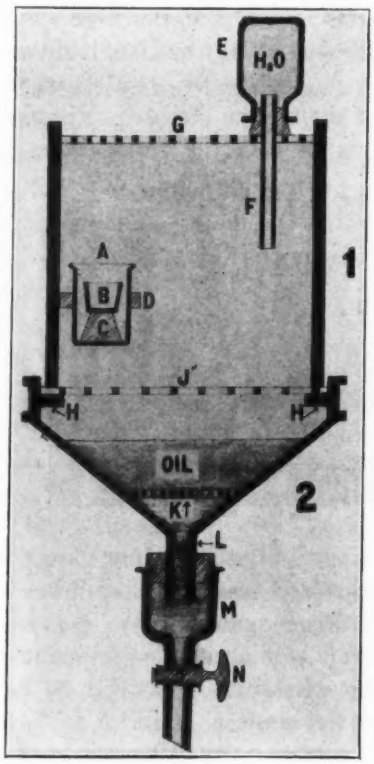

Fig. 1.

Fig. 2.

Fig. 1.—Diagram of metabolism cage (⅛ actual size).

Part 1. *A*, feeding device; *B*, food cup; *C*, rubber stopper; *D*, metal ring; *E*, water bottle; *G*, screen.

Part 2. *H*, lugs to support screen (*J*) and Part 1; *J*, screen on which animal rests (allows both urine and feces to drop through); *K*, screen to retain feces; *L*, stem; *M*, separatory funnel; *N*, stopcock.

structed of galvanized iron, with a diameter of 9″ across its top. The upper cylindrical cage (Part 1) fits snugly into it, resting on four small lugs (*H, H*). Between the upper and lower parts of the cage is a wire screen (*J*) which supports the animal. The mesh of this screen is large enough, ⅜″ mesh, to permit both the urine and feces to drop through into the mineral oil directly below.

The sides of the funnel taper down, with an angle approximately 30°, to the stem (*L*) 4½″ in length. This stem, with an inside diameter of ½″,

leads into the dropping funnel (*M*) which is attached by means of a tightly-fitting rubber stopper.

Mineral oil fills the entire funnel up to a level ½″ from the wire screen (*J*). Urine and feces drop through this screen, through the mineral oil as far as the small wire screen (*K*), which in turn retains the feces and allows the urine to flow down into the funnel (*M*), which contains a few cubic centimeters of 3 per cent sodium fluoride as preservative.

Collection of Urine.—At the end of the experimental period—usually of four days' duration—the urine is run off by means of the stopcock (*N*). To increase the accuracy of collection, a small glass plate is placed on the scale when the animals are to be weighed. Any urine voided during weighing can then be washed back into the cage. Inasmuch as no urine stains were observed on the sides of the upper section (I), only the screen (*J*) and the converging sides of the lower section (II) need be washed down with distilled water in an effort to obtain the last traces of urine. Finally, the washings are run off and combined with the urine sample which is kept in the refrigerator until

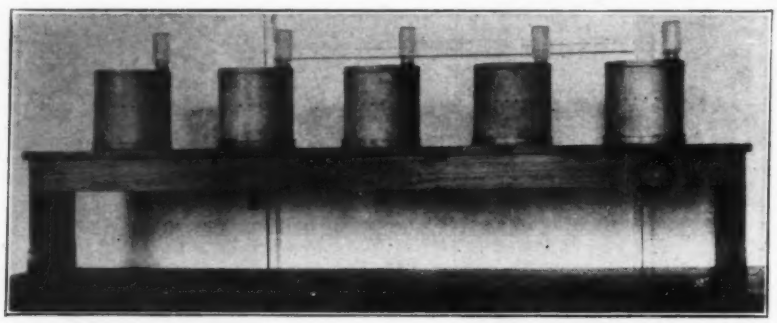

Fig. 3.

ready for analysis. After removal of the urine and washings, the mineral oil is drawn off, filtered from feces, sterilized, and used again.

Fig. 2 shows the cage in assembled form. In actual experiments with animals, a series of five cages is kept in a long wooden section (Fig. 3) having holes, 8¾″ in diameter, into which each cage fits closely.

Adherence to the measurements of the cage as given is necessary for its successful use in metabolism experiments.

Tests of Accuracy.—It is apparent that if the device described is to be used in metabolism experiments, the degree of accuracy of the cage in the collection of various urinary constituents must first be determined.

Tables I and II show the efficiency of the cage using acetone, nitrogen and a mixture of "acetone bodies" as test substances. Acetone was determined by the method of Van Slyke[11] and nitrogen by the Kjeldahl method. In all the experiments, the solution to be tested was trickled down the sides of the lower section (Fig. 1, Part II) and allowed to remain in the apparatus for four days. At the end of this period, the solution was run off and, together with the washings, was analyzed.

It was desirable first of all to ascertain the efficiency of the mineral oil itself in preventing volatilization of acetone. In Experiment 1, therefore, the

screen (*J*) was removed from the cage and an acetone solution was trickled down through the oil alone. This experiment shows clearly that no acetone was lost, testifying to the efficacy of the oil in the collection of urinary acetone.

Experiment 1 demonstrates the capacity of the oil to prevent volatilization of acetone. In actual metabolism experiments, the animal rests on the screen (*J*) and it was deemed advisable to determine what loss of acetone, if any, might be due to the presence of the screen. Accordingly, an acetone solution was trickled down over the screen (*J*) and allowed to drip into the oil directly below. Experiment 2 shows that when this procedure was carried out, a relatively large loss of acetone occurred, whereas the data for Experiment 1 indicate complete recovery. This loss may therefore be attributed to volatilization due to the presence of the screen (*J*).

In metabolism experiments with the rat, however, the value of 19 per cent obtained in Experiment 2 must be discounted considerably, when it is realized that in the experimental procedure no attempt was made to imitate the rat's habit of urination. Instead, the acetone solution, was trickled from a pipette in such a manner that *all* parts of the screen (*J*) were wet with the fluid. The rat, on the other hand, probably does not behave in this manner, but remains stationary on the screen and urinates directly through its wide meshes. It is obvious, therefore, that Experiment 1 more nearly approximates the actual experimental condition and undoubtedly 19 per cent is not the true index of the usual error of the cage in the collection of acetone but rather represents a maximum value.

Experiments 1 and 2 were concerned with only one of the three "acetone bodies," i.e., acetone. It was desirable next to find out, firstly, what losses the other "acetone bodies," i.e., aceto-acetic acid and beta-hydroxybutyric acid, would undergo in the cage and, secondly, to ascertain what loss acetone itself would suffer when all three "acetone bodies" are present in one solution as they are in urine. In the experiment referred to in Table II a mixture of all three "acetone bodies," made up in the approximate proportions excreted by the rat* was used. The solution contained 30.3 mg. of "acetone bodies" as follows: 4.2 mg. acetone, 7.2 mg. aceto-acetic acid (expressed as acetone) and 18.9 mg. beta-hydroxybutyric acid (expressed as acetone). Such a mixture, when trickled over the screen (*J*) into the oil suffered a loss of 9 per cent. Further analysis of the data in Table II shows that of this loss, 5.7 per cent was due to acetone and aceto-acetic acid, whereas beta-hydroxybutyric acid was responsible for the remaining 3.3 per cent loss. The data in this experiment also show that the sum of the acetone plus aceto-acetic acid present in the mixture accounts for a loss of 14 per cent, whereas beta-hydroxybutyric acid suffered a loss of only 5 per cent. Here again, the values are probably maximal ones.

In Experiment 3 (Table I) a sample of dog's urine, when trickled showed a loss of 3.1 per cent in nitrogen. Osborne and Mendel[2] report losses of 10 per cent or more in the collection of urinary nitrogen. Mitchell[10] by his method obtains an error of 2 to 3 per cent. The small error obtained with

*Unpublished data from this laboratory.

TABLE I

TESTS OF ACCURACY OF URINARY COLLECTION
ACETONE AND NITROGEN

EXP. NO.	URINARY CONSTITUENT	AMOUNT USED MG.	AMOUNT RECOVERED MG.	RECOVERY PER CENT	LOSS PER CENT	REMARKS
1	Acetone	6.84	6.84	100	00.0	25 c.c. of solution used. Screen (*J*) removed.
2	Acetone	9.50	7.70	81	19.0	25 c.c. of solution used. Screen (*J*) in place.
3	Nitrogen	427.6	414.4	96.9	3.1	20 c.c. of dog's urine used. Screen (*J*) in place.

TABLE II

TESTS OF ACCURACY OF URINARY COLLECTION
"ACETONE BODIES"

DETERMINATION*	AMOUNT USED		AMOUNT RECOVERED mg.	PERCENTAGE RECOVERY		PERCENTAGE LOSS		REMARKS
	mg.	Per Cent of Total Acetone		Of Each Constituent	Of Total Acetone	Of Each Constituent	Of Total Acetone	
Total acetone	30.3	100	27.6		91		9	Used 40 c.c. of a solution containing: Acetone = 4.2 mg. Aceto-acetic Acid = 7.2 mg. Beta-hydroxybutyric Acid = 18.9 mg. "Total Acetone" = 30.3 mg. 62.3% of the "total acetone" bodies present as beta-hydroxybutyric acid.
Acetone + Aceto-acetic Acid	11.4	37.7	9.7	87	32	13	5.7	
Beta-hydroxybutric Acid	18.9	62.3	17.9	95	59	5	3.3	

*Results of determinations of "acetone bodies" are expressed as acetone.

the cage described above suggests its value in metabolism experiments involving urinary nitrogenous substances.

SUMMARY

1. A metabolism cage is described which permits the collection of the urine of the rat. This cage is suitable for the study of ketosis in small laboratory animals such as the rat.

2. In the collection of urine, loss of volatile urinary substances is prevented by the use of mineral oil under which the urine is held.

3. The degree of accuracy in the collection of urinary "acetone bodies," i.e., acetone, aceto-acetic acid and beta-hydroxybutyric acid, as well as urinary nitrogen has been determined.

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STUDIES IN LOCAL ANESTHESIA. II

The Pharmacology of Some Para-Amino-Benzoate Compounds*

By H. McGuigan, S. J. Cohen, W. J. R. Heinekamp, et al., Chicago

INTRODUCTION

THE following series of para-amino-benzoate preparations were prepared and furnished to us by Roger Adams of the Department of Chemistry of the University of Illinois.

Series A

NAME	FORMULA	MOL. WT.
I Dimethylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(CH_3)_2.HCl$ $C_{11}H_{17}O_2N_2Cl$	244.5
II Diethylamino ethyl para-amino-benzoate hydrochloride (Procaine)	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_2H_5)_2.HCl$ $C_{13}H_{21}O_2N_2Cl$	272.5
III Di-n-propylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_3H_7(n))_2.HCl$ $C_{15}H_{25}O_2N_2Cl$	300.5
IV Di-n-butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(n))_2.HCl$ $C_{17}H_{29}O_2N_2Cl$	328.5
V Di-isopropylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_3H_7(iso))_2.HCl$ $C_{15}H_{25}O_2N_2Cl$	300.5
VI Di-isobutylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2(C_4H_9(iso))_2.HCl$ $C_{17}H_{29}O_2N_2Cl$	328.5
VII Di-secondary butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(sec))_2.HCl$ $C_{17}H_{29}O_2N_2Cl$	328.5

Series B

NAME	FORMULA	MOL. WT.
I Diethylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N.(C_2H_5)_2HCl$	286.6
II γDi-isopropylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N(\text{iso propyl})_2HCl$	314.6
III Di-normal propylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N.(\text{N.propyl})_2HCl$	314.6
IV Di-normal butylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N(\text{N.butyl})_2HCl$	342.7
V γDi-iso-secondary butylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N(\text{sec. butyl})_2HCl$	342.7
VI γDi-isobutylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N(\text{iso butyl})_2HCl$	342.7

*From the Laboratory of Pharmacology, University of Illinois, College of Medicine, Chicago.

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The corresponding B or propyl series was also furnished. This series differs from the A or ethyl series by being propyl para-amino-compounds instead of ethyl para-amino-compounds.

The local anesthetic effect of these drugs was determined by a number of methods, and while we do not consider any single method entirely satisfactory, they give information that cannot at present be procured by more desirable means. These methods have been previously used and justified by other investigators. The object of the investigation is the study of the nature of nerve impulse and anesthesia.

Anesthetic Value as Determined by Instilling Drug into Conjunctival Sac of a Rabbit's Eye

By S. J. Cohen, M.S., M.D., Chicago

METHOD

THE hair around the eye was clipped short to lessen the probability of touching or stimulating it during the experiment. The conjunctival sac was then filled with the anesthetic solution by retracting the lower lid, forming a pocket and filling it with the solution. This was allowed to remain in contact with the cornea for one minute. The excess of the anesthetic was then removed by allowing the eyelids to contract to their normal position. The point of a toothpick, wrapped in cotton, was applied to the cornea to test the winking reflex at one-half minute intervals.

An attempt was made to find a stimulus that could be stated in units (weight), but no satisfactory method could be devised. Since, however, the method used was accurate within a minute or two, we think it is quite sufficient.

The solutions used were of equimolecular concentration, and corresponded to 1 per cent procaine.

As a standard of comparison, the time required for the induction of anesthesia, likewise its duration, was determined in the following eight dilutions of cocaine: 4 per cent, 1 per cent, ¾ per cent, ½ per cent, ¼ per cent, ⅛ per cent, 1/16 per cent, 1/32 per cent.

These results are rather interesting. The onset of anesthesia of the various dilutions of cocaine was all within the time of application of the drug, i.e., one minute. The duration of anesthesia, with solutions up to

Table I

The Average Results with Each of the Dilutions

Cocaine HCl	Duration of anesthesia (in minutes)
4 per cent	48.00
1 " "	42.00
¾ " "	38.00
½ " "	23.30
¼ " "	17.00
⅛ " "	10.00
1/16 " "	5.00 (incomplete anesthesia)
1/32 " "	No anesthesia produced

1 per cent, varies directly with the concentration—the higher the concentration, the longer the duration of anesthesia and vice versa. However, there is no prolongation of anesthesia after a concentration of about 2 per cent. The only difference observed with the stronger solutions is the toxic effect of the drug on the cornea, as shown by drying, pitting, corrosion, etc. In concentrations lower than $\frac{1}{16}$ per cent there is practically no anesthesia induced so far as the rabbit's eye is concerned. Since the time of onset of anesthesia is the same with all these concentrations, it is clear that the time of onset cannot be used as a criterion of the anesthetic power of the solution.

Studies were then made with the ethyl-para-amino-benzoate compounds. The method used was as described above. One per cent novocaine was used as the standard (No. 2) and the other solutions were used in a corresponding molecular weight solution.

TABLE II

THE AVERAGE RESULTS OF THE NORMAL COMPOUNDS

DRUG	MOL. WT.	DURATION OF ANESTHESIA (IN MINUTES)
I	244.5	No anesthesia
II	272	14.00
III	300.5	16.00
IV	328.5	30.00

The isocompounds also act in relation to their molecular weight, but they are more toxic and irritant than the corresponding normal compounds. Table III shows the serial action.

TABLE III

ETHYL SERIES

DRUG	MOL. WT.	DURATION (IN MINUTES)
V	300.5	23
VI	328.5	45 (irritant)
VII	328.5	46

Studies were then made on a second series of anesthetics of the propyl-para-amino-benzoate compounds—six in all. Table IV gives the average results.

TABLE IV

DRUG	MOL. WT.	DURATION OF ANETHESIA (IN MINUTES)
I	286.6	26
III	314.6	35
IV	342.7	42

TABLE V

PROPYL SERIES

DRUG	MOL. WT.	DURATION (IN MINUTES)
II	314.6	60
VI	342.7	Too irritant for use
V	342.7	63

The results of these experiments are practically in the same order as the first series of drugs, except that in the propyl series the molecular weight is greater, and the increase in the duration time of anesthesia is correspondingly longer. When the anesthetic action of the para-amino compounds is

compared with the action of cocaine hydrochloride, their anesthetic efficiency on the rabbit's eye, arranged in terms of cocaine, is as shown in Table VI.

TABLE VI
COCAINE EQUIVALENTS
ETHYL SERIES

DRUG	
I	No anesthetic power
II	3/16% cocaine
III	1/4%
IV	5/8%
V	7/16%
VI	2%+
VII	2%+
PROPYL SERIES	
I	1/2% cocaine
II	4%+
III	3/4%
IV	1%
V	4%+
VI	Very irritant

From these results the occlusion may be drawn that the anesthetic efficiency of this homologous series of preparations in the rabbit's eye is proportional to the increase in the length of the normal chain, and to the increase in the molecular weight of the drug. Since these solutions were compared in molecular equivalents, and the molecular weights range from 244.5 to 342.7, there is a correction which must be made if a strict comparison of percentage solutions is desired. The molecular weight of procaine is 272.5. This was made in 1 per cent solution. If, therefore, one wishes the actual percentage comparison figures, it may be computed as follows:

272.5 : net weight of unknown :: 1 : x.

The molecular weight of cocaine hydrochloride is 339.65, so that no decidedly unfair ratio is obtained by assuming that molecular and percentage solutions are, for practical purposes, the same.

The toxicity of all these drugs, except No. IV in A series, is less than cocaine, and but slightly greater than procaine. They may, therefore, be used as local anesthetics in such regions as the eye. In the A series, Nos. V and VII seem especially worthy of consideration. No. VI, because of its caustic action, should not be used. In the propyl series, Nos. II and V deserve especial consideration. No. VI is too irritant for use.

THE STANDARDIZATION OF LOCAL ANESTHETICS BY THEIR APPLICATION TO THE SCIATIC NERVE TRUNK OF A FROG*

BY L. W. SCHULZ, D.D.S., CHICAGO

AFTER considerable experimentation, we have selected the following method: Frogs of approximately the same weight (25 grams) were decapitated just posterior to the optic lobes and before use were allowed to

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rest for at least two hours to recover from the shock. This period of rest is necessary to avoid marked difference in the response to the stimulus.

The frog was fastened to a cork plate by means of pins passing through the lower jaw and toes of the hind legs. A constant minimal stimulus from an inductorium was then applied to the webbed portion of the foot by means of a small electrode. This gave a positive contraction of the muscles anterior to the anesthetized area on that side of the body. This reaction was found positive in all the frogs tested.

The skin was now opened in a straight line for about two centimeters on the lower third of the dorsal surface of the thigh. This incision was made in the groove between the muscles. The fascia was then carefully separated by blunt dissection and the muscles pushed aside, permitting a full exposure of the sciatic nerve. Small hooks mounted in the ends of glass rods make very good instruments for this purpose. The muscles, skin, and fascia were retracted by small retractors made from safety pins, and the nerve exposed so that one centimeter of it could be immersed in the anesthetic. This leaves the nerve exposed in the bottom of a small diamond-shaped trough or well formed by the muscles, with one centimeter of the nerve anesthetized.

The anesthetic (procaine 1 per cent in physiologic salt solution) is now put into this well by means of a dropper. The foot of the frog is then stimulated every ten seconds after the anesthetic has been in contact with the nerve for three minutes, when there is no response (contraction) of muscles anterior to the anesthetized area on this side. This is the "sensory end point" and its average time is about *five minutes* for anesthesia. The "motor end point" is determined by stimulating the nerve trunk direct, central to the anesthetic, and getting a contraction of the gastrocnemius muscle. The average time for the motor paralysis was found to be 14.1 minutes. This stimulation of the nerve direct is not desirable, but any other form of stimulation central to the anesthetic gives a marked contraction of other muscles higher up in the leg and trunk, and this obscures the end point.

DETERMINATION OF COCAINE VALUES

The time for complete anesthesia with cocaine solutions was determined by the method already described. The results are:

COCAINE	AVERAGE TIME FOR ANESTHESIA
4%	0′ – 37″
2%	1′ – 39″
1%	2′ – 4″
½%	2′ – 30″
¼%	4′ – 31″
⅛%	8′ – 5″

From ten to sixteen trials were made for each average reading in order to reduce the error. The results were then plotted on graph paper and the results obtained from the series of para-amino compounds compared with it.

DETERMINATION OF PARA-AMINO BENZOATE VALUES

The solutions used were of equimolecular concentration, corresponding to one per cent procaine. The A series of drugs, seven in number, produced sensory anesthesia in the following time:

A	I	Average	time	13′ – 57″
A	II	"	"	5′ – 30″
A	III	"	"	1′ – 42″
A	IV	"	"	1′ – 30″
A	V	"	"	1′ – 5″
A	VI	"	"	3′ – 55″
A	VII	"	"	2′ – 0″

These average times also were obtained from ten to sixteen experiments with each drug.

These solutions compare with cocaine as follows:

A	I =	1/40%	cocaine	sol.
A	II =	1/6%	"	"
A	III =	1.7%	"	"
A	IV =	2%	"	"
A	V =	3%	"	"
A	VI =	1/4+%	"	"
A	VII =	2%	"	"

These results indicate the onset of complete anesthesia only. If the anesthetic is applied topically, its effect will not last as long as it will if the nerve is blocked by injecting the solution into the tissues. The time of onset of anesthesia is governed by the strength of the anesthetic, and the duration is governed by the power of absorption, destruction, or neutralization of the agent by the animal.

THE STANDARDIZATION OF THE B SERIES OF PARA-AMINO COMPOUNDS

This series of anesthetics was standardized the same as the previous groups, viz., on the sciatic nerve trunk of frogs. The inductorium was set at 8 cm. with a straight secondary coil. Stimulus was applied to the skin on the foot of the frog every five or ten seconds after the approximate time for anesthesia was determined. The temperature of the room was about 23° C. and the frogs were kept moist for about two hours after decapitation.

The results for the B series of local anesthetics were as follows:

B	I	Average	time	of	sensory	anesthesia	2′ – 32″
B	II	"	"	"	"	"	3′ – 0″
B	III	"	"	"	"	"	2′ – 8″
B	IV	"	"	"	"	"	1′ – 15″
B	V	"	"	"	"	"	1′ – 53″
B	VI	"	"	"	"	"	2′ – 20″

These solutions compare with the following strength of cocaine according to the curve plotted in previous experimentation, as follows:

B	I =	approximately	a 0.5%	cocaine	solution.
B	II =	"	a 0.4%	"	"
B	III =	"	a 0.9%	"	"
B	IV =	"	a 2.7%	"	"
B	V =	"	a 1.3%	"	"
B	VI =	"	a 0.7%	"	"

This method of comparison and standardization should be carried out at different intervals of a few days to a few weeks or months to see if these drugs will have the same anesthetic effect after aging.

Judged from the results of this method, Nos. III, IV, V, and VII of the ethyl series are very efficient local anesthetics, also Nos. IV and V of the propyl series.

Table I shows the results obtained on twenty frogs with one per cent procaine:

TABLE I

FROGS	TIME FOR SENSORY ANESTHESIA		TIME FOR MOTOR PARALYSIS	
	Right Leg	Left Leg	Right Leg	Left Leg
1	5½	5¾	16½	12½
2	5½	5¼	11½	14
3	5½	6	13¼	15½
4	4½	5	11	13½
5	4	5¾	15¼	16
6	5	4½	14½	15
7	6	5½	15	16¼
8	5¼	4½	11¾	16¾
9	4¾	5¼	16½	25¼ ?
10	5¼	6	13	14¼
11	5½	5	15	16¼
12	5	5¾	13	13½
13	5¾	5¾	16½	17
14	4½	4¼	15¾	16½
15	4	4½	15½	16
16	4	3¾	15	16
17	4¼	4	14½	15
18	5	4¾	15	14
19	4½	5	14	15¼
20	4¾	4½	15½	16
	Sensory Average 5 Min.		Motor Average 14.1 Min.	

DISCUSSION

The method outlined permits the study of local anesthetics in situ. Trauma and shock are reduced to a minimum. The nerve is immersed in the anesthetic in a manner which closely approximates the application of local anesthetics in actual practice. It permits the study of both sensory and motor paralysis and gives a valuable method of evaluating local anesthetics. A possible objection that may be brought against this method is that the muscles are also bathed by the anesthetic. This is unimportant because after sensory and motor nerves are both paralyzed, the muscles still react to direct stimulation. There is therefore no reason to suspect that the action on muscle is important or that the strength of the anesthetic bathing the nerve is changed to any important extent.

From the experiments carried out as outlined, the following conclusions may be drawn:

I. Absorption of the anesthetic has no apparent effect.

II. Sensory paralysis occurs before motor paralysis.

III. The anesthetic and paralytic power of a chemical series increases with the increase of molecular weight of the substituting alkali radicles. Ratio of the time of sensory to motor paralysis by this method is about 1 to 3.

The Relation Between the Time for Paralysis of the Sensory and Motor Fibers of a Nerve by Various Local Anesthetics as Determined by Their Action on the Sciatic Nerve of the Frog

By O. V. Pawlisch, B.S., Chicago

PRACTICALLY all methods of determining the time of paralysis in the frog's sciatic nerve by local anesthetics refer to motor paralysis. In the present investigation I have endeavored to find the relation between the time of sensory and motor paralysis, and whether this relation varies with different anesthetics.

Ostlund, Hodges, and Dawson[1] report experiments showing the effect of heat block on the sciatic nerve of the frog when the nerve was exposed and carried transversely through a rubber tube containing heated saline solution. They found that as the temperature was raised the sensory fibers were paralyzed first, but they have no definite time ratio between sensory and motor paralysis. Hafemann[2] had previously performed similar experiments with corresponding results.

The basis for the greater susceptibility of the sensory fibers is not known. The idea that it is due to a superficial situation of the sensory fibers is not sustained by the work of Kraus and Ingham,[3] and our discovery that not all the local anesthetics (notably apothesine) paralyze the sensory first confirms this observation. There remains then the possibility of a difference in chemical composition. It may be due to a difference in the lipoid solubility coefficient or in a lipoid difference in the composition of sensory and motor nerves, as discussed by R. Gottlieb[4] in his article "Pharmacological Investigation Concerning the Isomers of Cocaine."

Our work was done on frogs, but we employed several methods to arrive at our conclusions, and each method with its results is given below. In all cases the animals were decapitated at the medulla and pithed backward to the foramen magnum. The best results were obtained with frogs which were allowed to rest an hour or two after the operation before tests were made.

Method 1.—The thigh muscles were cut away, leaving only the sciatic nerve connecting the leg to the body. The frog was placed on a paraffin block and the nerve was led through a raised cistern, 0.5 cm. in diameter, filled with the anesthetic. Stimulation was produced by a constant definite faradic current, with the secondary coil at 3. The end points were as follows: For sensory—stimulation of the nerve trunk peripheral to the block and production of crossed reflex or twitching of body. For motor—stimulation of nerve trunk central to block, and production of contraction of calf or foot of the corresponding leg.

This method has some disadvantages, notably the possible injury to the nerve by direct stimulation, exposure to the air, and the fact that end points

TABLE I

RESULTS BY METHOD 1

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS OCCURRED IN	MOTOR PARALYSIS AFTER	SENSORY MOTOR RATIO
13	No anesthetic	17′ 9″	40′ 35″	42%
11	0.25% Cocaine	11′ 43″	22′ 7″	53%
12	0.50% "	4′ 58″	11′ 23″	43%
14	1% "	1′ 40″	3′ 17″	50%

were faint and in some frogs absent. A significant result is that the sensory paralysis occurs first, even without the use of an anesthetic, the nerve being merely exposed to the air. It is interesting to note that some of these experiments were performed during very hot weather in July, and some during cooler weather in October. During the hot weather the S./M. ratio was 38 per cent and during the cooler weather 51 per cent. This seems to be in accord with the work of Ostlund, Hodges, and Dawson on heat paralysis.

Method 2.—A pocket was made in the thigh by spreading the muscles around the sciatic and holding them apart with a clip, the nerve being exposed for about one centimeter of its length. The frog was placed on a cork plate and pinned down, the pocket being filled with the anesthetic. End points: Sensory—stimulation of the skin over the toe with faradic current and production of contraction or twitch of body of other leg. Motor—stimulation of skin over lumbar cords and contraction of calf or foot.

TABLE II

RESULTS BY METHOD 2

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
6	0.50% Cocaine	2′ 28″	11′ 30″	21.7%
6	1% "	1′ 54″	6′ 20″	30 %

This method has the advantage of avoiding the exposure and injury to the nerve which occurred in Method 1. It has the same disadvantage of no sharp sensory end point, and also that the stimulation over the lumbar cords was not exactly analogous with the sensory stimulation of the skin of the foot. This we believe accounts for the prolonged motor paralysis time.

Method 3.—Frogs were sensitized by a dose of strychnine just sufficient to produce a crossed reflex without severe tetanic convulsions (0.10 to 0.15 c.c. of a 0.01 per cent solution). A thigh pocket was made as in Method 2, but arranged so that when the frogs were suspended by the Türck Method the anesthetic would not run out. End point: Sensory—toe of blocked leg

TABLE III

RESULTS BY METHOD 3

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
4	1% Cocaine	2′ 12″	5′	44%

placed in 0.5 per cent H_2SO_4, and withdrawal of both legs. Motor—immersion of toe of unblocked leg, and the raising of both legs.

The results corroborate closely those of Method 1, but the method is attended with difficulty. An advantage is that the stimulus may easily be kept constant.

Method 4.—Frogs were sensitized with strychnine as in Method 3, but placed on cork plate as in Method 2. End points: Sensory—faradic stimulation of skin of toe in blocked leg and production of contraction in opposite leg. Motor—faradic stimulation of skin of toe of unblocked leg, and production of contraction in blocked leg.

TABLE IV

RESULTS BY METHOD 4

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
5	1% Cocaine	3′ 8″	6′ 12″	50.5%
4	1% B-Eucaine	7′ 11″	13′ 41″	52%
6	1% Butyn	5′ 34″	6′ 25″	87%
5	1% Apothesine	12′	11′	110%
5	1% Novocaine	7′ 45″	14′ 30″	53%
2	A-1	Not anesthetic		
4	A-2	8′ 37″	13′ 22″	64%
5	A-3	3′ 09″	3′ 48″	82%
6	A-4	3′ 15″	6′ 05″	53%
5	A-5	5′	6′ 54″	72%
4	A-6	6′ 37″	6′ 30″	101%
4	A-7	7′ 45″	8′ 45″	88%
4	B-1	12′ –7″	14′ 07″	85%
4	B-2	8′ 30″	9′ 52″	86%
6	B-3	5′ 50″	6′ 35″	88%
5	B-4	5′ 24″	6′	90%
5	B-5	9′ 06″	11′ 06″	82%
4	B-6	8′ 37″	9′ 45″	88%

CONCLUSIONS

1. That when a nerve is exposed to the action of various local anesthetics, the sensory and motor fibers are not paralyzed at the same time.

2. That for each local anesthetic there is a fairly definite ratio between the time for motor and the time for sensory paralysis.

3. (A) That cocaine, B-eucaine, butyn and novocaine paralyze the sensory fibers first; (B) That apothesine paralyzes the motor fibers first. (Details in Table IV.)

4. That this selective action is not due to a superficial location of the sensory fibers.

5. That under exposure to heat and drying, the sensory fibers lose their power of conduction before the motor fibers as they do under anesthetics.

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USE OF A SUCTION APPARATUS FOR BLEEDING RABBITS*

By C. M. Anderson, M.D., C.P.H., Toronto, Canada

THE three methods most commonly employed in bleeding rabbits which have been successfully immunized against a specific antigenic substance are:

(1) Cutting the marginal ear vein.
(2) Severing the vessels of the neck.
(3) Aspiration from the heart by the use of a syringe.

The third method is probably the method of choice with most laboratory workers on account of the ease with which the blood may be obtained in a sterile condition. It is, however, sometimes extremely difficult to obtain more

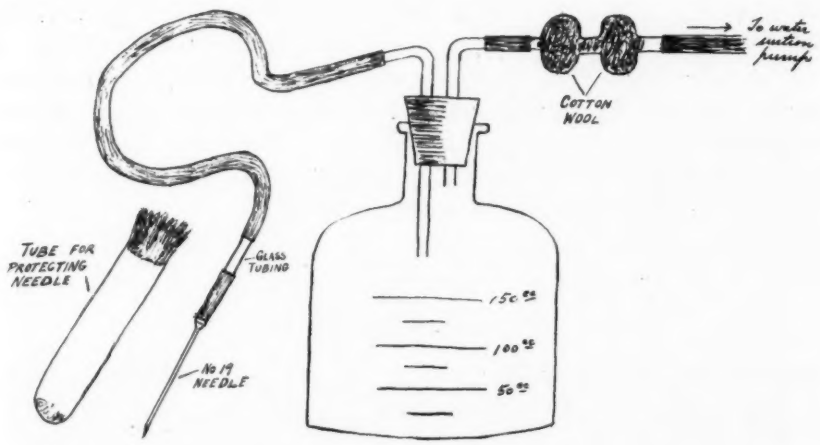

Fig. 1.

than 40 or 50 c.c. of blood by this method. A syringe with a capacity of 30 c.c. is about as large as can be conveniently handled in bleeding rabbits from the heart. When the syringe becomes filled, the operator must either withdraw the needle from the heart or disconnect the needle from the syringe and leave the needle inserted into the heart while the contents of the syringe are transferred to a sterile centrifuge tube. I have frequently seen experienced workers produce a satisfactory titer in a rabbit but fail to obtain more than 30 to 40 c.c. of blood.

With the apparatus described and illustrated, one can practically exsanguinate the animal with one puncture of the heart. Yields of from 90 to 100 c.c. are easily obtainable.

We make up about six of the above apparatus at one time. These are wrapped in cotton towelling and sterilized in the autoclave for one-half hour

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at 15 pounds. Those not used in a month's time are again sterilized. By this practice we have the apparatus constantly available.

It is essential to use a good sharp needle entirely free from rust or other partial obstructions. The only difficulty encountered with the apparatus in our laboratory has been due to lack of attention to this detail.

The rabbit is etherized, the hair over the cardiac area clipped short, and the skin painted with tincture of iodine. The apparatus is attached to a water suction pump and the operator inserts the needle into the heart over the point of the cardiac impulse. When the heart has been entered, the blood will be observed to flow up through the glass window into the collecting flask. The needle is held in position until the last drop of blood is obtained.

Upon completion of the bleeding, the blood is allowed to stand one hour at room temperature for clotting to take place. The rubber stopper and connections are then replaced by a sterile cotton plug and the flask is placed in the refrigerator overnight to allow the serum to separate completely. The following morning the serum is removed from the clot. Usually only a small portion of serum will require centrifuging. The serum is then mixed with an equal volume of C.P. glycerin and dispensed into vials of 5 c.c. capacity.

The apparatus may also be used for obtaining sterile defibrinated rabbit blood for enrichment of media. When the apparatus is used for this purpose, glass beads are placed in the flask and while the bleeding is proceeding, the flask is gently shaken to prevent coagulation. The rabbit need not be bled out unless desired. The defibrinated blood is stored in the cold room and used as required to make blood agar slants or plates.

The apparatus has been in use for over a year with consistently good results.

IMPROVED FORMS OF SEVERAL COMMON LABORATORY APPLIANCES*

By J. P. Quigley and W. C. Heath, Augusta, Georgia

SEVERAL pieces of apparatus used in the biologic sciences have recently been developed in this laboratory. They appear to be superior to those formerly used and are so easily made that it seems advisable to publish a description of them.

Frog Heart Four-Way Perfusion Tube.—This consists of a block of brass with holes drilled at right angles to each other. Brass tubes are screwed into the holes and the tubes connected to reservoirs containing the perfusion fluid. Fig. 1 (C). The apparatus is easily cleaned, does not get out of order, is very strong and holds a small amount of fluid.

Signal Magnet.—This apparatus is made from an electric door bell by removing the frame containing the magnets, removing the striker ball and

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also one of the magnets, see Fig. 1 (B). The wires are so arranged that the current does not flow through the interrupter but instead makes a permanent magnet. This signal is decidedly more durable and has a stronger movement than those now on the market.

Drop Recorder.—The foundation of the recorder is a Harvard heart lever. A cup made of cork and paper coated with shellac is attached to the lever. The cup has perforations around the edge to permit the escape of the fluid.

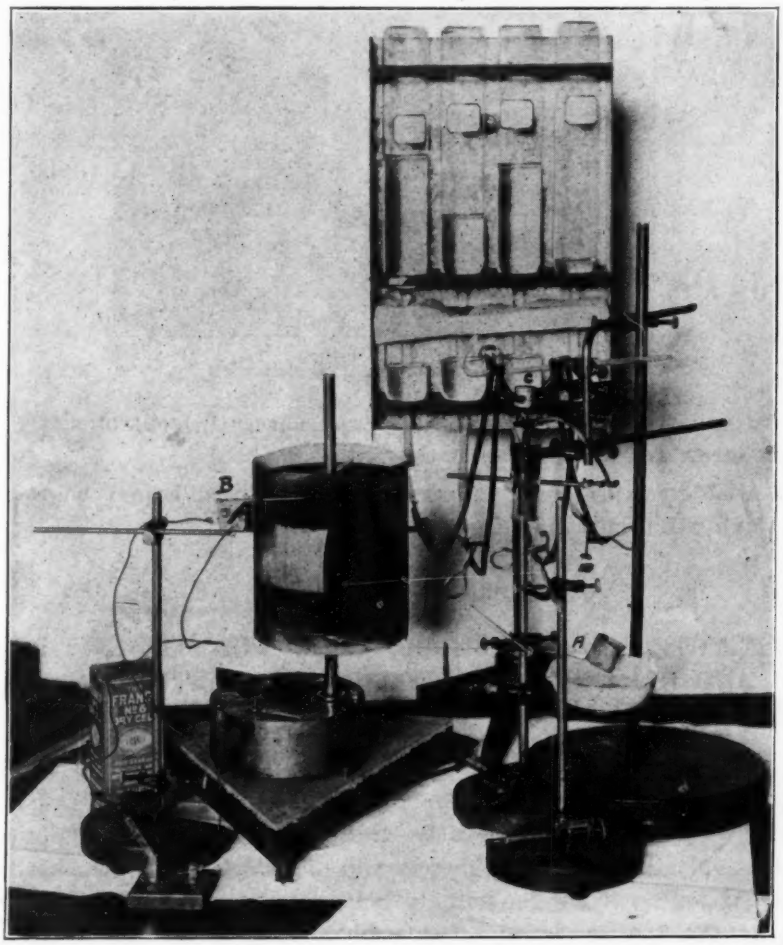

Fig. 1.—One of the possible uses of (*A*) drop recorder, (*B*) signal magnet, (*C*) 4-way heart perfusion tube.

A piece of wire fastened to the bearing support of the heart lever projects for about an inch towards the writing end of the lever. A small piece of dental dam connects the lever and this piece of wire, see Fig. 1 (A). This recorder is easily made, is inexpensive, and rarely requires attention.

Anesthesia Regulator.—For anesthetizing animals when a tracheal cannula is employed, this apparatus permits of an unusually accurate control of the rate at which the anesthetic is given and when the stage of surgical anesthesia is attained becomes almost automatic in its operation. This appa-

ratus is made from the strainer of a percolator and an automatic oil cup. A piece of sponge is placed inside the strainer and the apparatus mounted on a plate or block of wood, see Fig. 2. Ether or chloroform is placed in the

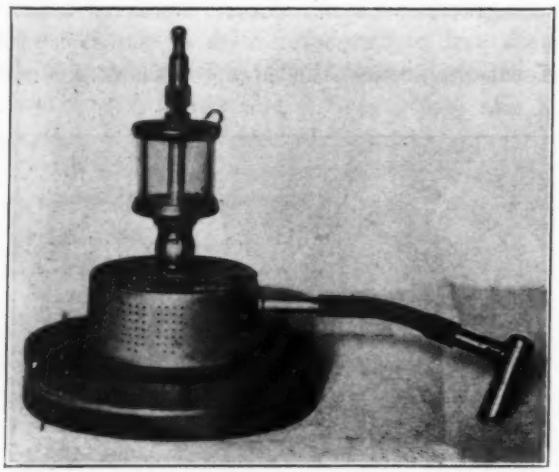

Fig. 2.—Anesthesia regulator.

glass cup and the rate of flow readily and carefully controlled by the adjustment screw and valve lever at the top of the cup.

We wish to express our appreciation to William Salant whose interest in this work has been of material assistance.

A SUBSTITUTE FOR THE RUBBER BULB FOR USE IN THE KOLLS AND ERLANGER BLOOD PRESSURE INSTRUMENTS*

By Lawrence S. Kubie, M.D., Baltimore, Md.

SATISFACTORY records can be obtained from either of these instruments only if one has a rubber membrane which is just sensitive enough, and neither too rigid nor too soft, to transmit the pulsations of the pressure system to the writing lever. The rubber bulbs which have always been used for this purpose are now very hard to obtain. They are no longer manufactured in this country; and large supplies cannot be imported because the fabric rapidly loses its elasticity. Therefore, we have devised a very simple and inexpensive substitute for these bulbs and have found it entirely successful. During the past winter so many inquiries have been received at this laboratory in regard to a possible source of the rubber bulbs, that it seems advisable to publish a diagram of this substitute.

The diagram is almost self-explanatory. Essentially the instrument con-

*From the Department of Physiology, The Johns Hopkins Medical School, Baltimore, Maryland.
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sists of a brass hemisphere instead of the glass bulb of the Kolls or Erlanger apparatus, and a rather large rubber tambour in the place of the rubber bulb. The hemisphere screws down over the tambour and when pressure is pumped into the cuff around the arm the rubber tambour is stretched until it nearly fills the cavity of the brass hemisphere. Its pulsations within the hemisphere then correspond to the pulsations of the rubber bulb within the glass bulb of the instrument in its usual form. We merely substitute the pulsation of a hemisphere of rubber within a hemisphere of brass for those of a sphere of rubber within a sphere of glass. The hemisphere can, of course, be made of glass, and can be sealed to the base plate with sealing wax. The rubber

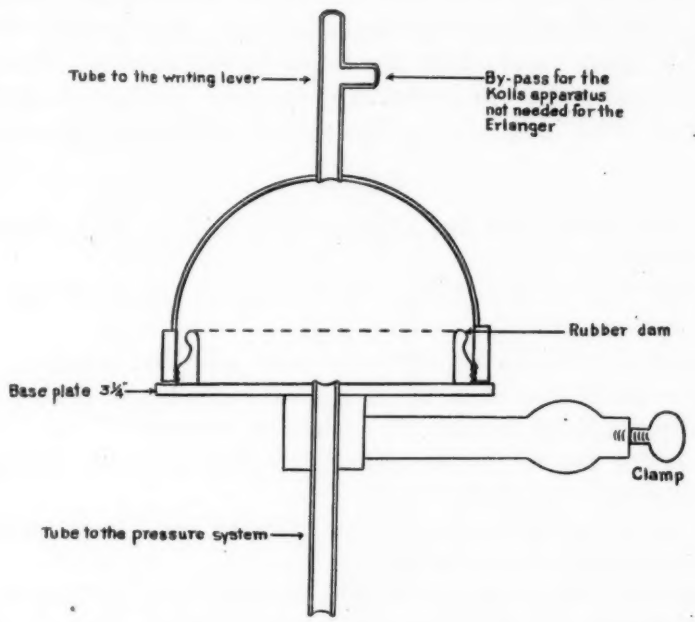
Tube to the writing lever
By-pass for the Kolls apparatus not needed for the Erlanger
Rubber dam
Base plate 3¼"
Clamp
Tube to the pressure system

Fig. 1.

dam that is used in the tambour will vary with the work to be done; any weight and tension can be used. For classroom work we have found that a double thickness of fairly heavy dam was more sensitive than the rubber bulbs and had less tendency to fling, giving far more consistent records than had been obtained with any rubber bulbs which we could secure.

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CONCERNING THE INOCULATION OF ANIMALS WITH SOLID INOCULUM*

By G. H. Hansmann, M.D., Iowa City, Iowa

TISSUE inoculations into animals for diagnostic purposes are frequently attended with difficulties, and the results are not always reliable in that they do not conform with the histologic findings. The greatest difficulty is encountered in those cases where the lesion is one attended with little necrosis or exudation and much productive reaction. We have devised the following method which is simple and has proved satisfactory in our hands.

METHOD

1. Materials needed are fine silkworm-gut, scissors, long cutting needle and tissue forceps.

2. The tissue is attached to the silkworm-gut which is in turn threaded into a long cutting needle.

3. Incision is made in the abdominal wall with the scissors.

4. The needle is entered in the abdominal incision and drawn out in the groin drawing the tissue against the abdominal wound.

5. The lower flap of the wound is retracted and with steady traction the tissue slips into the groin.

6. The remaining silkworm-gut may then be used for a stitch or two in the wound of the abdominal wall.

The ease with which this procedure is accomplished, the lack of objection shown by the animal, the small amount of tissue injury and the rapidity with which healing takes place are all remarkable. The pain shown by the animal is much less than that resulting from the building of pockets in his anatomy in which to place the tissue not mentioning the insecurity of the collodion-cotton pocket seals and what happens to the tissue when the pockets are inverted. The tissue inoculated by the above method is held firmly in place and the tract taken by the inoculated tissue seals immediately and in from two to three days is healed quite firmly.

The tissue applicable to this type of inoculation is synovial tissue, bone and quiescent tuberculous lesions in which much fibroblastic reaction is present. It is in these types of lesions that the discrepancy between histologic and animal inoculation results is greatest and also where a positive animal is of greatest value.

We believe that the procedure outlined is an improvement on methods commonly employed in the inoculation of human tissues into animals for diagnostic purposes.

*From the Laboratory of Pathology, University Hospital, Iowa City, Iowa.
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A SIMPLE DEVICE FOR HASTENING FILTRATION THROUGH BERKEFELD FILTERS*

By F. A. Stenbuck, M.S., New York City

THE filtration of slow filtering colloidal solutions through filters of the Berkefeld type may be expedited where both air pressure and vacuum are available by the use of a simple device illustrated in the diagram. A piece of brass pipe about seven inches long is threaded at both ends and pro-

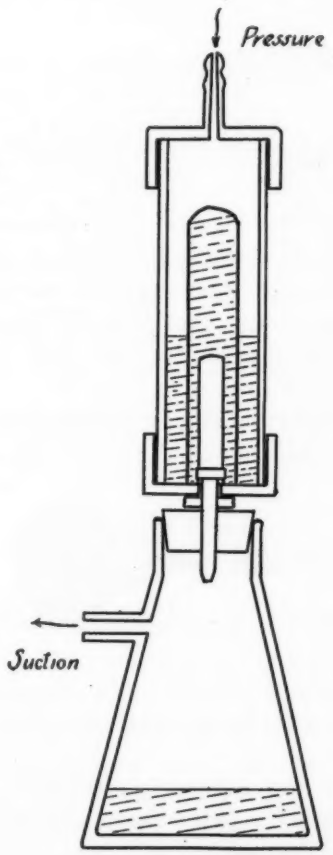

Fig. 1.

vided with brass caps. A three-eighths inch hole is drilled in one cap. The other cap is drilled through and provided with a brass stopcock nipple. A rubber ring gasket is placed in each cap to make a tight fit.

In using the device the stem of the filter candle is passed through the

*From the Pathological Laboratory, Mount Sinai Hospital, New York City.
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hole in the cap and tightened with the usual lock nut on the outside. The cap is screwed onto the pipe tightly and the solution to be filtered is placed in the pipe, as in the mantle of the usual filter. A test tube large enough to fit loosely over the filter candle is filled with the solution and inverted over the candle to insure the use of the whole candle surface while there is any liquid left in the mantle. The top cap is screwed on tight and connected to the source of compressed air. Both suction and pressure may then be used at the same time. Filtration may be further speeded up by opening the apparatus when the flow of liquid through the filter stops, and removing the film of slime from the surface of the candle with a small brush, such as a toothbrush.

In this way we have reduced very considerably the time necessary to filter solutions containing heavy suspensions of bacterial débris.

TO DRY AND CLEAN HYPODERMIC NEEDLES*

By Walter M. Brunet, M.D., Brooklyn, N. Y.

THE care of hypodermic needles is, at best, a disagreeable task and oftentimes after a hypodermic or other injection, the needle is laid aside to be cleaned and dried later. Usually when the job of cleaning the needle is reached it is found to be clogged up. Oxidation is the foe of all steel needles and this destructive power is due to the fact that needles are put away without being properly dried.

The simple inexpensive instrument here illustrated is for the purpose of drying and cleaning hypodermic needles.

Fig. 1.

It consists of a B-D Asepto rubber bulb, ½ ounce capacity, into the neck of which is inserted a male Luer adapter. This adapter fits all Luer needles.

To use this instrument, the adapter is inserted into the hub of the needle and the bulb compressed several times. In this manner, the in and out action of the air through the needle will blow out the moisture and dry it thoroughly.

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EDITORIALS

Blood Platelets and Leucocytes in Tuberculosis

IN 1914, some students of tuberculosis at Colorado Springs reported the following observations:[1, 2, 3]

1. At an altitude of 6,000 feet, blood platelets are increased in the circulating blood of man and guinea pigs. The average of counts made on one hundred healthy young men at sea level (New York) was 302,000; at 6,000 feet (Colorado Springs) 340,000, an increase of about 12 per cent.

2. Blood platelets are consistently increased in tuberculosis of man and guinea pigs. The counts in healthy guinea pigs were found to average somewhat over 400,000; in tuberculous animals somewhat over 6,000,000. In man the findings were classified:

(Normal 340,000)					
Early afebrile tuberculosis,	45	counts,	35	patients	— 368,000
Advanced quiescent tuberculosis,	50	"	40	"	— 412,000
Advanced active tuberculosis,	26	"	22	"	— 496,000

Quite recently Bannerman[4] has reported a similar series of observations made at Montana in Switzerland, at an altitude of 5,000 feet. His platelet count on normal individuals (number not stated) varied from 250,000 to 380,000, the average being 300,000. He does not report any counts on normal persons at sea level by his technic, which is different from the methods commonly used. He made counts on 65 cases of pulmonary tuberculosis, and found, as did the workers at Colorado Springs, "that the blood-plates are generally present in excessive numbers in active pulmonary tuberculosis, and that, broadly speaking, the more serious the clinical condition the greater the degree of thrombocytosis."

As to the application of these facts, Bannerman writes, "It appears that there exists an inverse relation between thrombocytosis and the subject's resistance to the disease; it is suggested that the plate count may afford a means of measuring that somewhat indefinite entity, which is the basis of prognosis."

The earlier investigators of the subject considered this possibility, but it soon became apparent that in tuberculosis those classes of patients who showed increase of platelets showed an approximately parallel increase of polymorphonuclear leucocytes; the platelet count was, therefore, deemed a superfluous addition to the routine examination of blood in this disease, and was given up.

It may be added that of all blood examinations, morphologic, serologic, and clinical, the one which is of the greatest use in the prognosis of tuberculosis is the differential leucocyte count. The facts, noted by Ullom and Craig[5] about twenty years ago, and since amply confirmed by many others, may be epitomized thus: The more polymorphonuclears, the worse the prognosis; the more lymphocytes, the better the prognosis.

In a paper[6] published in 1923, the results of over 2000 counts on some 400 tuberculous patients at Colorado Springs were analyzed and tabulated thus:

	"Lymphocyte index"
I. 100 normal adults	43
II. 130 arrested or approximately cured cases	45
III. 120 cases improving	37
IV. 100 cases failing	24
V. 40 cases progressing to death	16

The "lymphocyte index" comprises the aggregate percentages of large and small lymphocytes, large mononuclears, and "transitionals." The so-called transitional cell is, of course, a form of the large mononuclear. In the light of our studies and those of others it would perhaps be better to omit these cells from the index, since they do not, like the lymphocytes, vary directly with the favorableness of the patient's condition. Our findings were as follows:

	Large mononuclears	
	Per cent	No. per cu. mm.
Normals	8	624
Arrested and cured cases	8	608
Far advanced active cases	6	840

McJunkin and Charlton,[7] in 1919, reported that these cells may be increased to as much as 10 per cent in advanced tuberculosis of guinea pigs, while in the normal animals they averaged 1.5 per cent. Further observations on this point have agreed with these, and it appears that in guinea pigs a high percentage of such cells indicates rapidly progressing disease, while in them, as in man, a high lymphocyte count indicates relatively effective resistance.

Like the erythrocytes and the platelets, the lymphocytes are increased at high altitudes. This fact, first reported in 1909,[8, 9] has been widely substantiated.[10, 11, 12, 13]

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—G. B. W. and C. T. R.

The "Face Value" of the Wassermann Report

LIKE all innovations in medicine the Wassermann test, so-called, has undergone many vicissitudes. At first heralded with acclaim, later criticized and even reviled in some quarters, it is only within recent times that this procedure has approached a proper evaluation; and, strange to say, the establishment of the complement-fixation test in syphilis in its proper relation to the diagnosis and treatment of the disease has been as much the result of the efforts and teaching of the serologist as of the strictures, comments, and criticisms of the clinician.

Despite the gradual appreciation of the true status of the complement-fixation test as only a single symptom, a phase of syphilis; as only one part of what should be an extensive, intensive, and exhaustive examination at times embracing many specialized forms of investigation; despite the emphasis properly laid upon the fact that the most important and vital feature of the Wassermann report should be its interpretation, serologists are still occasionally exhorted to improve their methods of examinations so that "reports may with safety be accepted at their face value."

Reduced to its ultimate significance this is really a demand that the complement-fixation reaction in syphilis be made infallible, that reports bear the significance and authority attributed to the Delphian oracle, that the diagnosis of syphilis be stripped of all complexities and difficulties other than those attendant upon the collection of a specimen of blood. The treatment would consist solely of the attainment of serologic negatives.

This, of course, is impossible of achievement nor will there ever be any method of laboratory procedure devised in connection with the diagnosis and treatment of syphilis which will relieve the practitioner of the necessity for the careful and minute study of each individual case.

It is unfortunate that the conception of biologic specificity upon which the original Wassermann test was founded still exerts a subconscious influence.

The first blind reliance upon a positive reaction as irrefutable evidence of syphilis was followed, as was to be expected, by an equal degree of belief in a multiplicity of conditions capable of giving a false positive reaction and it is only within very recent times that the high degree of relative specificity of the test has been revealed by the painstaking investigations of numerous workers.

Refinements of technic have added much to the delicacy of the test so that the reacting substances may be detected and even measured when present in remarkably small amounts. The reagents and manipulative factors of the technic have been modified and refined to such an extent that the reliability of a positive reaction has been tremendously increased.

Much has been done, therefore, to enhance the "face value" of a positive report—but nothing appears possible of early achievement which will warrant the hope of infallibility for the Wassermann report in general.

It must always be remembered that an estimate of the reliability of a report is inseparably associated with an understanding of the reliability and delicacy of the technic whereby it has been attained. No two reports may safely be compared without a coincident comparison of the merits of the technic or methods involved.

The "face value" of a negative report remains simply this: it is a statement of failure to detect in the serum examined at the time of examination any bodies reacting with a syphilitic "antigen." It applies to the conditions obtaining only at the time of examination; it bears no relation to past conditions and holds no guarantee for the future. Its proper evaluation is entirely dependent upon the proper summing up, and the individual and collective evaluation of all the circumstances pertinent to the particular case.[1]

The true explanation of the complement-fixation test in syphilis remains to be elucidated. In so far as is known it is the result of an interaction between the tissues and the invading spirochetae. Obviously, therefore, the production of reacting bodies—best spoken of collectively as reagin—is directly dependent upon: (a) the activity of the spirochetae and their effect upon the tissues; and, (b) the ability of the tissues to react.

The detection of reagin, when produced, is directly dependent upon: (a) the amount in which it is produced, and (b) the delicacy of the method in use.

Consideration of these basic principles makes clear the reason for the occurrence of negative reactions in syphilis. They are due, either to a

[1]Kilduffe, R. A.: The Status of the Negative Wassermann, Jour. Am. Med. Assn., Dec. 30, 1922, lxxix, 2215.

quiescent state of the spirochetae in which their effects upon the tissues are reduced to a minimum, to a lack of ability on the part of the individual to react, or to a failure of the individual to produce reagin in amounts capable of detection by the method of complement fixation in use.

Certainly it is true that in undoubted cases of syphilis negative reactions occur and, what is not readily susceptible of explanation, such negative reactions may occur in patients exhibiting clinical evidence of active lesions and may follow within a relatively short time a previous positive reaction.

Such instances are common to the experience of both serologist and clinician and have been reported, though it must be said that the possibility of their occurrence is better appreciated by the serologist.

Under these circumstances, therefore, the acceptance of a Wassermann report at its "face value" indicates a lack of understanding of the factors influencing the occurrence and detection of the reaction, and an equally serious lack of appreciation of the essential necessity for a careful study of each case upon its individual merits.

By methods in ordinary use, except that described by Kolmer, a positive reaction may be obtained in pregnancy in the absence of syphilis, especially when the cord blood is tested, in the febrile stage of pneumonia, in leprosy, and in yaws. The only nonluetic condition so far reported as giving a false positive reaction with Kolmer's quantitative technic is yaws.

By all the methods in common use, including Kolmer's, negative reactions may be obtained which do not represent the absence of syphilis, though with the latter technic the frequency of such false negative reactions is reduced to a minimum.

The "face value" of the Wassermann report, therefore, is simply that of a single, isolated examination. Its true value depends upon its correlation with and interpretation in the light of all the other findings: clinical, historical, and laboratory, of the individual case at hand.

—*R. A. K.*

The Periodic Health Examination

THE principles of public health were inaugurated in their crudest form long before the dawn of civilization, when savages, fearing the demons of disease, segregated and shunned the sick. The ancient Mosaic laws were in many respects models of public health and excellent applications of the principles of preventive medicine. Indeed, many applied directly to *individual* health and hygiene. Relics thereof still are seen in some portion of the modern church ritual. Mohammedans, for whom their prophet formulated similar great laws of personal hygiene, have maintained many in more nearly the original form. Thus, the Mohammedan obeisance which must be repeated at least thirty times daily, is an excellent exercise which in the Christian church has devolved into the simple genuflection. The cleansing of the entire body has but a relic in the modern dipping into holy water.

The recognition of the contagiousness of many diseases and the necessity

for protecting society against individual illnesses led to the establishment of public health measures which have developed until they, today, form the vast science of preventive medicine. While public health regulations benefit society as a whole, the individual also profits. Not infrequently *individual* preventive medicine has been applied in the past. Examples are antirabic treatment, the prophylactic administration of tetanus or diphtheria antitoxin, and vaccination against smallpox and typhoid fever.

The possibility of applying preventive medicine for the betterment of the individual was recognized decades ago. About 1870, Dobell, in England, suggested periodic examinations for life insurance companies. In 1900, Gould, of Philadelphia, read a paper before the American Medical Association urging the institution of health examinations by the family physician. Barés, in France, in 1902, discussed the possible value of periodic examination of healthy or apparently healthy persons.

In 1909, the Provident Life Insurance Company adopted the suggestion that medical examinations be made every five years. The Life Extension Institute of New York was organized in 1914 by a group of laymen and has since made over 250,000 examinations. Periodic examination of employees of the health department of New York City was begun in 1914. The annual examination of army officers was instituted in 1922. The National Health Council, a lay organization, desired to begin a campaign for yearly health examinations in 1922 but this was postponed for one year because the American Medical Association felt that physicians were not yet ready to cooperate in such a campaign. The National Health Council did institute its campaign in 1923, two years ago, with the slogan, "Have a Health Examination on Your Birthday." The American Medical Association endorsed the movement in 1922 and reiterated its endorsement in 1923 and 1924.

The Association first brought it to the attention of the medical profession in January, 1923, in an article by Dodson, and in May of the same year a concrete program for examination was presented in an article by Emerson. Since this time the idea has been put into practice by physicians in many of the larger centers and several articles dealing with the subject have appeared in the literature.

While individual preventive medicine deals chiefly with the prevention of metabolic, infectious, or other disturbances arising within the body, it also entails increasing of the bodily resistance against infection from without, either by promotion of general hygienic measures or by specific immunization. The aim is the establishment and maintenance of good health. As Tobey points out, good health is considerably more than mere disease dodging. "It is the ability so to live that the maximum benefit is derived from living. It is not only getting along without being ill, but it is that condition in which one is able to enjoy life. It is the perfect regular operation of the human machine so that there is the greatest productivity, utility, and potentiality."

In applying the principles of preventive medicine, we attempt to recognize chronic disease in its incipiency, and by clearing up predisposing causes such as focal infection, constipation, overeating, etc., to prevent or retard the progress

of its development. Indeed, in those cases in which disease has not yet made its appearance but in which certain faulty habits, errors of diet, etc., clearly predispose, we may point the way to right methods of living so that even the beginnings of disease may better be avoided.

The method of the health examination is essentially that used in the presence of obvious disease but the examiner's attitude is altered in that he must recognize the significance of minor abnormalities, determine as to their relative importance, and realize their potentiality for subsequent evil.

The examination must be most painstaking and comprehensive, not only for the gaining of the patient's confidence, but so that we may be sure that the earliest pathologic changes have not been overlooked.

With the increasing complexity of modern civilization and its great dynamic urge, problems of individual health have arisen which were unknown to the ancient physician, practicing on a more nearly vegetative race. It is a weak point in our practice that while aiding the patient with outspoken disease, we are often unable to relieve the below-par individual, who knows that all is not well within his economy, but in whom no definite organic abnormality can be discovered. It is within this enormous group that the cultist, the Christian Scientist, the osteopath, the chiropractor, the Abramite, makes such tremendous inroads. Their self-confidence, the "promise of cure," and the absence of serious progressive constitutional disease often combine to give remarkable symptomatic benefit, which, however, is often of short duration.

Indeed, many of these charlatans are more experienced in instilling principles of right living than is the busy practitioner who finds it less time-consuming to write an offhand prescription for some drug of doubtful potency. Modern pharmacology has cut the drugs of proved worth to a remarkably small number. The giving of drugs outside this small list may have some temporary benefit but since they do not remove the true cause of the patient's distress, the results are no more lasting than those obtained by the cultist. True, the wholesale use of drugs in the past makes it difficult to convince the patient that health may be acquired through hygienic reconstruction, often without adjunct medicinal therapy, and I consider it often thoroughly legitimate to prescribe tonic medication, but this is of little value unless accompanied by explicit and detailed instructions in personal hygiene, the clearing up of chronic infection, etc. "The physician of today should be not a dispenser of pills but a purveyor of positive health." (Bill.)

There exists a great group of degenerative and metabolic diseases which, once developed, remain resistant in greater or less degree to all therapeutic measures. These can be attacked only in their incipiency or in predisposed cases by personal preventive measures.

Who is best qualified to advise and supervise the patient? Today, it is the visiting nurse, the school nurse, the social service worker, the life insurance company, the institution organized and directed by laymen, the health chat in the daily press, the "home journal," and so on, which bring the message of hygiene to the individual.

Pioneer work is now being accomplished through "Hygiea." The one per-

son fully qualified to advise in the individual case, who alone is acquainted with the patient's physical make-up, his tendencies toward emotional reactions, his antagonisms, his proneness or not to morbid anxieties, and who has intimate knowledge of the patient's domestic environment, his habits, his familial tendencies, his hereditary predispositions is the family physician. The physician, so qualified, is the appropriate person to direct the patient regarding his health and his personal hygiene. It is he alone who is qualified through experience to know what to emphasize and what should be minimized. Individual health, like community health, is the province of the physician and other organizations, many of which are of undoubted intrinsic worth, must necessarily serve as adjuncts and work in collaboration with the directing physician.

However, until the physician realizes this, equips himself properly to perform the function of health adviser and asserts his prerogative, the present situation will continue. What is the present situation? The health promotion institute makes a more or less detailed examination, gives a fairly comprehensive and apparently satisfactory report to the patient, and then advises, if any abnormality is found, that he consult his own physician for specific instruction. As it now stands, then, the problem comes back to the physician, but it is to him that it should have been originally presented. On the other hand, the patient, after examination by physicians working for a lay organization, receives the report that all is well, loses contact with his own physician, and contents himself with an annual repetition of his health study. Often some intrinsic unadjusted problem, such as a problem of the home which is known only to the family physician, remains undiscovered and undiscussed and later may give rise to trouble.

Then there is another type of examination given by nonmedical organizations; annual or periodic urinalyses. It is surprising what a large group of successful business men submit themselves for this examination and upon receiving a negative urinalysis report, settle back thoroughly content in the knowledge that they have been pronounced sound in every respect. They have been paying from ten to twenty-five dollars annually for a procedure which alone is of doubtful worth.

The average duration of life has been increased from forty to fifty-eight years within the last seventy-five years. This increase has been accomplished almost entirely through reduction in infant mortality and deaths from contagious disease in the earlier years of life. It is confined almost entirely to age groups under thirty-five. Above forty-five, there are more deaths per thousand than there were twenty years ago. During the last century there has been practically no improvement in mortality among the aged. With existing knowledge we cannot hope to increase the normal life span much beyond the biblical limit of three score years and ten. As yet medical science is unable to prevent the degenerative changes associated with senescence.

However, with the knowledge which we already possess, properly applied, we can accomplish much in delaying degenerative changes, particularly of the heart, kidneys, and blood vessels. Dublin has constructed a hypothetical life table which expresses the best mortality we may hope for with our present

knowledge. In it the total expectation of life at birth is sixty-five years. This means an addition of eight years to the life span now prevailing in the United States. Of course, a great deal of the improvement will result from decreased mortality in the earlier years of life, but the factor of earlier recognition and preventive treatment of chronic, infectious, metabolic, malignant, and degenerative diseases, plays a very definite part.

About 30 per cent of accepted life insurance risks supposed to be practically normal, succumb to cardiovascular diseases.

Three conditions: Heart disease, Bright's disease and cerebral hemorrhage, curtail the present expectation of life by about four years. These deaths represent heavy loss to the community because they often involve people still in the prime of life and at the height of their usefulness.

The productive period of man rarely extends beyond the age of sixty-five. It is our hope that the average duration of life may be extended through this period of productivity, but we must bear in mind that the application of individual preventive medicine is not designed only to decrease the *death rate* but also, and particularly, to prevent disability in those who, while still living, are made inefficient and unhappy by disease.

The extent of unrecognized chronic disease is surprisingly high. Ninety-one members of the Kings County Medical Society, as an experiment, submitted to health examinations. The ages ranged from thirty-six to fifty years. Eighteen per cent of the ninety-one had minor defects requiring hygienic correction or minor dental or surgical attention. Eighteen per cent required medical supervision as well as hygienic correction and 4.5 per cent had advanced physical impairment requiring systematic medical or surgical care.

Craig reports a health survey of 5,706 police and firemen of Philadelphia. Nearly 2 per cent had some serious cardiac disease, over 2 per cent pulmonary tuberculosis, 5 per cent had albuminuria and nearly 1 per cent showed glycosuria. The majority of these men considered themselves well. Twenty-four per cent were overweight by 20 per cent or more, 2 per cent had bilateral nasal obstruction, 17 per cent had diseased tonsils, and over one-fourth had obvious dental defects. Arterial hypertension with systolic blood pressure 160, or over, was present in nearly 6 per cent of these supposedly normal individuals.

Of the 3,764,000 young men examined for the draft, 47 per cent had physical defects of sufficient importance to note on the reports of the examination, and 21 per cent were found physically unfit for all military service.

In 1909, the Metropolitan Life Insurance Co. began an active campaign of health education among its millions of industrial policyholders. This was supplemented with nursing care of those who were acutely ill. The death rate dropped about one-third in the next ten years. There was an increase of eight and one-half years in the life expectancy. Tuberculosis has declined 50 per cent among those insured in the Metropolitan Life Insurance Co. This is better than the general decline.

We must acknowledge that little has been accomplished for the patient after defects have been discovered, unless the patient is given proper advice toward remedying them or toward mitigating their evil results. This requires a detailed

study, not only of the patient's physical condition but of his daily routine, habits, and hygiene. That in practice the individual does profit by having such an examination is indicated by the statistics just quoted. One insurance company which has offered free physical examinations to its policyholders, with advice based upon the examination, calculates that already there has been a return and a profit of 200 per cent on their investment, due to savings in mortality. If a business organization finds this much value in the giving of free medical service to its policyholders, surely a routine physical examination should have a corresponding value to the individual. This value would be further increased when the service was individualized.

The educational value and the tremendous opportunity to the family physician for promulgating health and hygiene are obvious. When a patient who has been thoroughly examined by his physician does develop some acute illness, the physician will undertake the treatment with much greater confidence because he is already acquainted with the terrain over which he must work.

While recognizing the undoubted value of periodic health examinations, we must at the same time realize certain dangers and limitations of the procedure. Perhaps the greatest of these is the risk of mental trauma to the abnormally introspective or neurasthenic, which might make invalids of previously healthy individuals.

A centipede was happy, quite,
Until a frog in fun
Said, "Pray, which leg comes after which?"
This raised her mind to such a pitch
She lay distracted in the ditch,
Considering how to run.

Walsh points out that Pasteur did some of his greatest work, years after his first stroke of apoplexy. Had his physician insisted upon a vegetative existence, after the first illness, the world would have lost much of what Pasteur was able to give.

Each case must be individualized and must be handled with a degree of discretion and judgment which the physician alone has been in a position to acquire.

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—*W. T. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*International Clinics, March, 1925**

THIS follows the customary procedure of the series by devoting articles to diversified features of the practice of medicine. The classifications found in the volume are *diagnosis* and *treatment, mental disturbances, surgery, toxicology* and *"progress of medicine for 1924."*

Dr. Barker's article on the treatment of the psychoneuroses is of interest for comparison with similar articles by the author written in years past and in tracing developments and changes in the author's method. Two articles on urologic problems and pyuria give helpful information concerning the simpler methods of urologic study which may be applied by the general practitioner. It is regrettable that these two articles appear to have been borrowed nearly verbatim from works of another author. Seventy odd pages have been contributed by Henry W. Cattell and Jas. F. Coupal in which they review the progress of medical science for the year 1924. This makes a very convenient reference appendix.

Chemistry of the Blood in Clinical Medicine†

THIS is a British contribution and reflects the British attitude toward our present problems of blood chemistry. It is not a laboratory manual but a monograph presenting the knowledge of today in a systematized form. One is impressed that the majority of contributions on this subject, particularly the pioneer work in blood chemical methods, originated in the United States.

The author first presents the chemical constitution of normal blood, followed by a concise, clearly presented study of acidosis and alkalosis. The chapter on blood sugar is presented in the usual manner but that on nephritis reflects more nearly the continental attitude. The author divides the nephritides into azotemic and hydremic. He discusses the protein metabolism and the salt and water metabolism in these two types. He presents only those tests of kidney function which are used by preference in England, namely, the Ambard coefficient and Addis' ratio. The phenolsulphonephthalein test is not presented. Chapters on tetany, rickets and gout are brief but to the point. "Anoxemia" is divided into the arterial type, stagnant type and

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*International Clinics. A quarterly of illustrated clinical lectures and especially prepared original articles. By leading members of the medical profession throughout the world. Edited by Dr. Henry W. Cattell, 1, 35th series, 1925, J. B. Lippincott Co. Philadelphia. Cloth. Pp. 301.

†Chemistry of the Blood in Clinical Medicine. By O. L. V. de Wesselow. M.B. (Oxon.) F.R.C.P. (London) Cloth. Pp. 255. Price $4.50. William Wood & Co. N. Y., 1925.

anemic type. Oxygen therapy is of course most satisfactory in the arterial type but the author observes that in the anemic anoxemia, oxygen administration may tide the patient over an emergency. Other pathologic conditions discussed are hydremia, anhydremia and glycemia.

A short appendix presents briefly the usually accepted laboratory methods.

The volume is a contribution of worth and should be of value to all those who directly or indirectly encounter the pathologic conditions which the author discusses.

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*An Approach to Social Medicine**

THE author presents a contribution to the science of social welfare which will be found to have great intrinsic worth. His viewpoint is that of the psychiatrist. He points out a number of glaring faults not only in present-day customs and repressive influences but also in many of the recent procedures in dispensing social service. "The amateur social doctors are like the amateur physicians—they always begin with the question of remedies and they go at this without any diagnosis or any knowledge of the anatomy or physiology of society."

The book in its broadest sense becomes an analysis of human character and human behavior, particularly in their relationship to the community. This analysis is primarily biologic so that as its exposition develops, we can clearly follow and understand abnormal mental and emotional reactions in the individual. It is on an understanding of the causes for these reactions that the author bases his suggestion for more intelligent treatment or control.

The sex impulse being one of the greatest driving forces, receives detailed consideration and the psychology of this impulse is followed as it changes from the lower animal through the cave man to the present day extremely complicated social machine. The book necessarily forces a more tolerant attitude upon the reader, but this alone would be not without danger were it not that the author completes his presentation by suggesting in a general way the proper procedure for dealing with the problems. "Of all avenues of approach to the conflict between reproductive and social tendencies the one offering at the present time the broadest perspective appears to be that through which youth is passing to take command of new positions developing on the sites of deteriorated traditions. Candidly this means the evolution of a closer comradeship between men and women, based on sexual desires more equally distributed between the sexes, with a more intensive physical development and a higher spiritual organization, casting into the discard the exploded notion that human sexual contact has for its sole purpose reproduction. While a single standard of morals is to be insisted upon for both men and women it must be recognized that at the present time the

*An Approach to Social Medicine. By Francis Lee Dunham, M.A., M.D., Lecturer on Social Medicine. Johns Hopkins University. Cloth. Pp. 242. Price $4.00. Williams & Wilkins Co., Baltimore, 1925.

sexes do not share this responsibility equally, the burden falling far more heavily on the continent male.

"Whatever contributes, therefore, to evolve from the 'ladylike' type of Victorian neurasthenic, whose deterioration was due largely to masculine ideas of feminine inferiority, a higher model of comrade, is worthy of widest adoption.

"Instead of segregating the semidependent members of society at state expense, a better policy now advocates their early individual training and continued guidance in the community. This plan shows favorable results when limitations imposed by inferior intellectual capacity are recognized socially and correlated with the individual's conduct capabilities.

"Unfortunately community assistance partakes too much of benevolence, sentimentally dispensed, or of coercion inducing pauperism, and it lacks efficiency in dealing with a social group that always may be semidependent and childlike. Recreation centers, the family wage system, social insurance and health protection, are undoubtedly more rational means of treating 'feeblemindedness' in its higher grades than segregation and sterilization."

At the opposite extreme the author asserts that individuals of unusually keen intellect are often stifled in childhood by standards of mediocrity and suggests that in the future these may be given greater opportunity for the exercise of their intellects.

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*General Systematic Bacteriology**

THIS work deals primarily with two phases of bacteriologic systematization, the classification and the nomenclature of microorganisms. The author stresses the difference between the two, remarking that while naturally interrelated they may be altered individually. He presents a brief history of the bacteriologic classifications so far proposed and proceeds to discuss the codes of nomenclature and their relationship to the problems of bacterial terminology. Following this is an alphabetical list of all of the names which have been used by various authors to designate bacterial subgenera, genera or higher groups. The classification suggested by the author is based in part upon the international botanical code.

This work differs from Berger's *Manual of Determinative Bacteriology* in two chief essentials. The classification recommended by Buchanan is not entirely the same as that recommended by Berger and his coworkers, and while Buchanan's book is a monograph on classification, Berger's is in addition a key to the identification of bacteria.

These two volumes represent the latest and most comprehensive efforts to present appropriate classifications of bacteria. Their finality must await the judgment of those bacteriologists who will weigh the practicability of their application.

*General Systematic Bacteriology, 1. By Dr. R. E. Buchanan, Iowa State College, pp. 597, Cloth. Price $6.00. Williams & Wilkins Co., 1925.

*A Laboratory Manual of Physiological Chemistry**

A PRACTICAL working manual in which are incorporated most of the recent so-called practical methods of biochemical analysis with the usual laboratory studies customarily made by undergraduate students in acquainting themselves with the properties of biochemical substances. Among the outstanding chapters which differ to some extent from the older undergraduate manuals are ones on the relation of the ionic theory to properties of living tissues and fluids, on vitamines and deficient proteins, on the acid-base equilibrium in the animal body, short chapters on the systematic testing of urine, classification of tests on blood and urine, and an appendix on the best methods for obtaining and preserving test materials.

While written primarily as a student's manual it should be useful as a reference work for the laboratory worker. The system of blood analysis is particularly comprehensive. The Folin-Wu system is detailed as well as methods for the determination of various other blood constituents as sodium, calcium, potassium, magnesium, phosphorus, chlorides, sulphates, acetone bodies, oxygen capacity and blood gases in general.

The Eastern Association of Tropical Medicine†

TRANSACTIONS of the fifth biennial congress at Singapore 1923. The unabridged transactions are classified into papers dealing with malaria, beriberi, ankylostomiasis, leprosy and miscellaneous. The majority of the malaria papers deal with its epidemiology and prevention. Occasional cases of insanity consequent on malarial infection are reported. These, however, are very infrequent. Several papers deal with the curious condition observed among the Malay peoples known as amok, a form of temporary insanity in which the individual suddenly runs about, killing all who cross his path. This is the derivation of the familiar expression "running amuck." It occurs chiefly among Malays, has been occasionally seen in foreigners who have settled on the Malay peninsula, but apparently does not occur in those Malays who have moved elsewhere. The condition appears dependent upon paranoia and paranoiac forms of insanity, upon acute mania and upon epileptic mental states. Latah, another psychosis observed particularly among Malay races, consists of a most unusual state of suggestibility, an acute hypnotic state.

Wu Lien Teh is inclined to the opinion that the original home of the plague is in the great plateau of Central Asia. It is carried endemically in the wild rodents of this region. He emphasizes the importance of the tarabagan, a small rodent first described by Marco Polo, as a carrier.

The variety of subjects covered and the high character of the contributions makes this a desirable volume for the student of tropical medicine.

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*A Laboratory Manual of Physiological Chemistry. By Elbert W. Rockwood, M.D. Ph.D., and Paul Reed Rockwood, M.D. Illustrated. Pp. 413. Fifth edition, Cloth. Price $4.00 F. A. Davis Co. Philadelphia, 1924.

†The Far Eastern Association of Tropical Medicine. Transaction of the Fifth Biennial Congress held at Singapore, 1923. Edited by Dr. A. L. Hoops and Dr. J. W. Scharff. Illustrated. Pp. 973, Cloth. John Bale Sons & Danielsson, Ltd., London, 1924.

*Handbook of Bacteriology**

THE author has set out to provide a handbook, particularly for students in which the so-called practical aspects are alone incorporated. He has succeeded well. The book is not purely bacteriologic in its treatment but covers phases of immunology and serology. Briefly, it may be described as a volume which covers in condensed form the field which is so comprehensively surveyed by Kolmer in his *Infection, Immunity and Biologic Therapy*. In the portion devoted to systematic bacteriology the alternative nomenclature is that recommended in Berger's *Manual of Determinative Bacteriology*.

The illustrations are unusually well done.

International Conference of Health Problems in Tropical America†

A COMPILATION of contributions, 71 in all, which taken in toto deal with nearly all phases of tropical medicine. The proceedings should be of value to all who are interested in tropical medicine. While epidemiologic and similar considerations find place in most of the articles the major portion is given over to clinical studies.

The Congress which met between July 22 and August 1, 1924, at Kingston, Jamaica, was the guest of the United Fruit Company and the proceedings have been published by this organization. This is a most laudable instance of the wider interests taken by private business enterprise not only in industrial hygiene but in its contiguous ramifications.

The report has been edited by Dr. M. J. Rosenau.

Laboratory Diagnostic Methods‡

THE book aims to "present in a concise manner the technic of the various laboratory tests without any discussion of their value, limitations, or clinical application in the diagnosis and treatment of disease." The first portion is a revision of Pepper and Coleman's *Manual on Pathology*. The present edition represents the clinical, pathologic, bacteriologic and serologic methods employed in the department of pathology and bacteriology of the Graduate School of Medicine in the University of Pennsylvania. It is designed essentially for hospitals and busy practitioners who desire brief yet comprehensive descriptions of the various tests and methods.

Most of the newer contributions have been incorporated. Such are the Rosenthal test for liver function, the Widal and Abrami liver function test, methods for estimating the digestive ferments in duodenal specimens, preparation of colloidal gold reagent, and manufacture of vaccines.

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*Handbook of Bacteriology. For Students and Practitioners of Medicine. By Joseph W. Bigger, M.D. (Dublin) F.R.C.P.I., D.P.H. Cloth. Pp. 413. Price $5.00. William Wood & Co., N. Y., 1925

†International Conference on Health Problems in Tropical America, held at Kingston, Jamaica, B.W.I. Pp. 1010. Cloth. United Fruit Company. Boston, Mass. Illustrated, 1924.

‡Laboratory Diagnostic Methods. By Dr. John A. Kolmer and Dr. Fred Boerner, Cloth. Pp. 338. D. Appleton & Co., N. Y., 1925.

The bacteriologic section deals with the practical laboratory diagnosis of the various bacterial diseases. The blood chemistry section is based on the Folin-Wu system. The section devoted to serology presents agglutination reactions, the Kolmer Wassermann reaction and the Kahn precipitation test.

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*The Chemical Aspects of Immunity**

DR. WELLS stresses that our progress in understanding the fundamental principles of immunity has been slow because we have on one side of the equation a living animal and on the other proteins, the most complex of all known chemical compounds. He suggests that the equation may be simplified by the use of purified proteins of a fairly well-known composition in place of such hopelessly complex mixtures as bacteria or blood serum. Not infrequently the immunologist is struggling with agents of unknown composition and is measuring his results with a yardstick of most uncertain accuracy. Or a physical chemist is applying methods of great accuracy to materials of uncertain nature and to reactions which are modified by an infinitude of unknowable variables.

The author presents concisely and rather briefly present knowledge of the phenomenon of immunity in such a way that it will be most intelligible particularly to students of chemistry. The work is rather didactic. Instead of being a brief for any one theory it presents all aspects of the problem with whatever critical interpretations appear necessary.

The subdivisions are as follows; antigens: immunologic specificity; the nature of antibodies; the neutralization of toxins by antitoxins; agglutination and precipitation reactions; lytic reactions; the Wassermann reaction and related reactions with syphilitic blood; hypersensitiveness—anaphylaxis—allergy; phagocytic immunity; resistance to nonantigenic poisons.

A helpful feature is that the author presents a recapitulation at the end of each chapter with the suggestion that these be read first, before more extensive study of the book is undertaken.

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*The Chemical Aspects of Immunity. By H. Gideon Wells, Ph.D., M.D. Cloth. Price $4.00. Pp. 254. The Chemical Catalogue Company, Inc., N. Y., 1925.

The American Society of Clinical Pathologists

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Dallas Meeting

Preparations are already being made for the coming convention next spring at Dallas. Members are requested to lay their plans early for attending the annual conclave which bids fair to equal, if not surpass, its predecessors. More and more the American Society of Clinical Pathologists is proving a tower of strength and a source of inspiration to all laboratory workers. Kindly notify the secretary if you desire to present a paper.

Dr. Spitz's Address Before the Clinical Congress of Surgeons

It has become a tradition with the American College of Surgeons to invite a representative of the American Society of Clinical Pathologists to read a paper at their annual meeting. The interests of the two organizations are closely interwoven; both are intensely desirous of elevating the scientific standards in hospitals. The proper conduct of the clinical laboratory involving important aids to diagnosis is an indispensable factor in the structure of hospital standardization. Dr. Spitz, who is a member of our Executive Committee, was delegated to address the Congress at the Philadelphia meeting, his article being "Pathological Conferences in Standardized Hospitals." The paper was received with hearty commendation and liberal discussion and will appear in the transactions of the Congress.

Society Notes

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.